Disruptive membrane interactions of alpha-synuclein aggregates

Iyer, Aditya; Claessens, Mireille M. A. E.

Published in:
Biochimica et biophysica acta-Proteins and proteomics

DOI:
10.1016/j.bbapap.2018.10.006

Publication date:
2019

BBA - Proteins and Proteomics 1867 (2019) 468–482

Contents lists available at ScienceDirect

BBA - Proteins and Proteomics

journal homepage: www.elsevier.com/locate/bbapap

Disruptive membrane interactions of alpha-synuclein aggregates

Aditya Iyer[a], Mireille M.A.E. Claessens[b,*]

[a] Membrane Enzymology Group, University of Groningen, Groningen 9747 AG, The Netherlands
[b] Nanobiophysics Group, University of Twente, Enschede 7522 NB, The Netherlands

ARTICLE INFO

Keywords:
Alpha-synuclein
Membrane
Amyloid
Interactions
Aggregation

ABSTRACT

Alpha synuclein (αS) is a ~14 kDa intrinsically disordered protein. Decades of research have increased our knowledge on αS yet its physiological function remains largely elusive. The conversion of monomeric αS into oligomers and amyloid fibrils is believed to play a central role of the pathology of Parkinson's disease (PD). It is becoming increasingly clear that the interactions of αS with cellular membranes are important for both αS's functional and pathogenic actions. Therefore, understanding interactions of αS with membranes seems critical to uncover functional or pathological mechanisms. This review summarizes our current knowledge of how physicochemical properties of phospholipid membranes affect the binding and aggregation of αS species and gives an overview of how post-translational modifications and point mutations in αS affect phospholipid membrane binding and protein aggregation. We discuss the disruptive effects resulting from the interaction of αS aggregate species with membranes and highlight current approaches and hypotheses that seek to understand the pathogenic and/or protective role of αS in PD.

1. From generic amyloids to amyloids of alpha synuclein

1.1. A time capsule; the discovery of alpha synuclein (αS) amyloids

The term “amyloid” as used today in the biochemistry and biophysics community, refers to fibrillar protein structures with a typical width of 5–10 nm that have a characteristic cross β-sheet secondary structure. Deposits of these fibrils in or outside cells are also called plaques and exhibit positive birefringence under polarized light. Less than two centuries ago, the normal starch-like constituent in plants that can be visualized using iodine staining was referred as amyloid [1]. Sixteen years later, intracellular deposits in brain tissues stained positively by iodine were therefore thought be carbohydrates and their relevance to disease was believed to be circumstantial [2]. It was in 1859 when Friedreich and Kekulé showed that amyloid plaques mainly contained proteins, that the research attention shifted to the study of amyloids as protein aggregates [3]. Subsequently, the presence of amyloids was thought to be a consequence of aging and disease conditions including cancer and many auto-immune diseases rather than a cause of disease.

In 1912, Friedrich Lewy described proteinaceous inclusion bodies in neurons of patients suffering from Parkinson's disease (PD) [4]. These spherical and thread like inclusions in neuronal bodies, now called Lewy bodies (LBs) and Lewy Neurites [5], would later be recognized as a pathological hallmark of PD. > 4 decades later, Cohen and Calkins showed, using electron microscopy, that the proteins in these inclusions had a characteristic fibrillar ultra-structure. The dimensions of the fibrils ranged between 5 and 12 nm in width and were referred to as amyloid fibrils [6]. Further studies showed that the protein fibrils, irrespective of their origin, were composed of even thinner structures which were named protofibrils [7,8]. The following year, the basic structure of the protein fibrils was shown to be a β-pleated sheet [9]. We shall henceforth refer to such fibrils as amyloids. Since then, numerous reports, using high resolution techniques like cryo-electron microscopy (Cryo-EM), solid state nuclear magnetic resonance (ssNMR), magic angle spinning nuclear magnetic resonance (MAS-NMR), x-ray fiber diffraction (XRD) and two-dimensional infra-red spectroscopy (2D-IR), have fueled the structural understanding of the amyloid state of numerous proteins (Fig. 1). It is now known that amyloid formation is not a rare phenomenon associated merely with diseases but rather it defines a structurally and thermodynamically stable form of proteins. The amyloid fibril is a low energy alternative to the native state, which can in principle be adopted by many, if not all, polypeptide sequences [10]. There are now about 50 known disorders with widely disparate symptoms, each of which involve the conversion of normally soluble and functional peptides/proteins that possess either a distinct secondary structure or are intrinsically disordered into amyloid fibrils [11]. If, and how, the transition to the amyloid state is a

* Corresponding author.
E-mail address: m.m.a.e.claessens@utwente.nl (M.M.A.E. Claessens).

https://doi.org/10.1016/j.bbapap.2018.10.006
Received 16 April 2018; Received in revised form 14 August 2018; Accepted 4 October 2018
Available online 11 October 2018

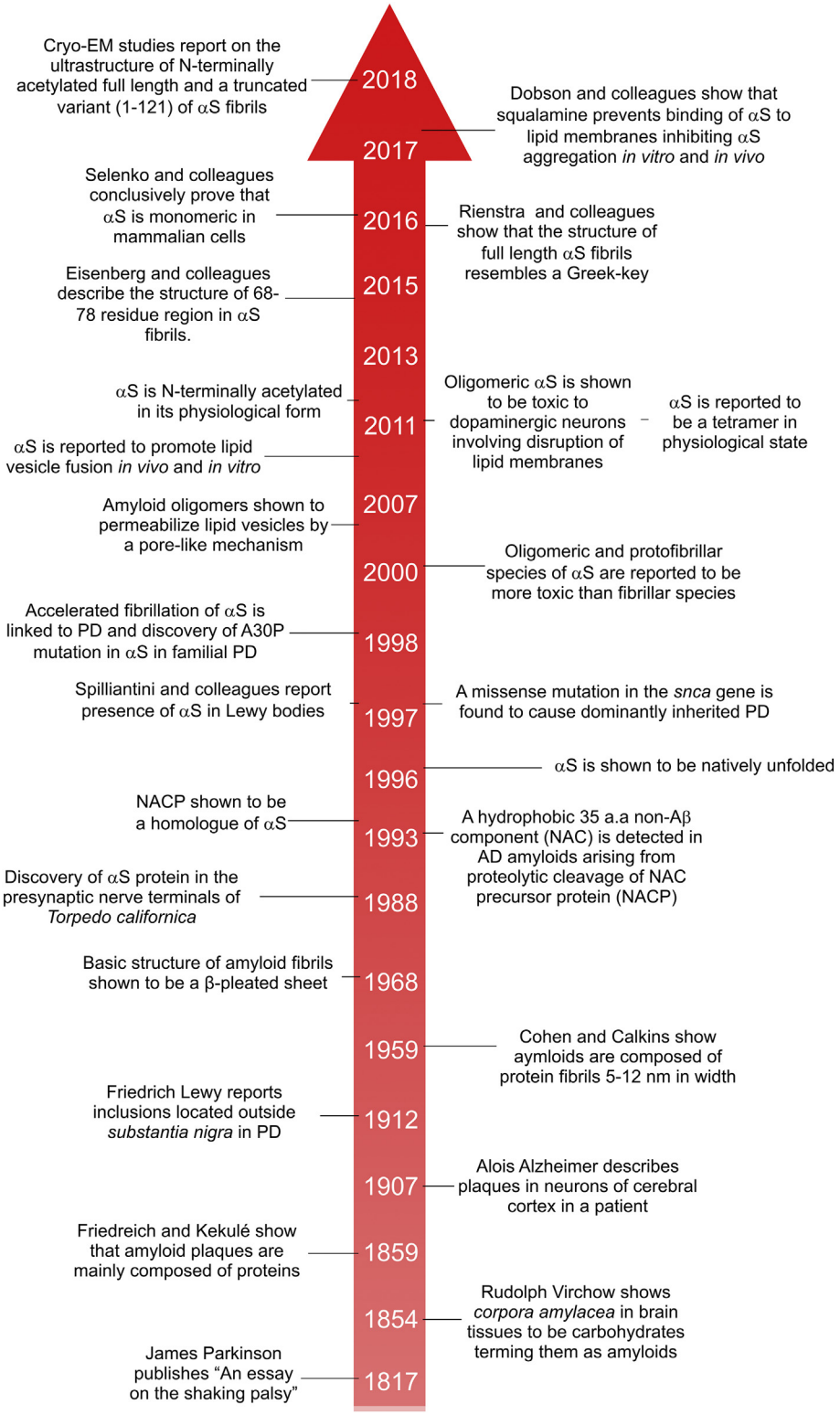

Fig. 1. Timeline of selected events relating to αS and amyloids.

consequence or a cause of these diseases is still debated. Here we will review the possible causative relation between the formation of amyloid aggregates of the protein alpha-synuclein (αS) and the development of Parkinson's disease. αS is a soluble intrinsically disordered protein (IDP) that is abundantly present in neurons. The protein is associated with intracellular membranes and membrane binding is one of its major putative functional roles. Membranes are however also implicated as the main target of toxic interactions. In reviewing the role of αS aggregates in the development of PD, we will therefore focus on the possible membrane damage caused by amyloid aggregation or aggregates.

The main protein found within amyloid deposits in LBs of Parkinson's disease patients is αS. This protein was initially found in the synapse and in the nuclear envelope of *Torpedo californica* [12] in 1988.

The connection of αS to neurodegenerative disorders was not established until the discovery of a distinct peptide component in the amyloid plaques in the brains of Alzheimer's disease (AD) patients [13]. This ~35 amino acid peptide component was referred to as the non-Aβ component (NAC). The NAC was generated from the proteolytic cleavage of a 140 amino-acid protein called NAC precursor protein, NACP. NACP was later shown to be a homologue of human αS [14,15]. The NAC peptide itself turned out to be highly amyloidogenic and antibodies raised against synthetic NAC peptides recognized amyloid fibrils in AD plaques [13,16]. NACP was subsequently described as a natively unfolded protein [17] that loosely associates with synaptic vesicles [15,18] and expresses abnormally in the presynaptic terminals of neuronal cells of the central nervous system in patients afflicted with AD [15,19] (Fig. 1).

The link between αS/NACP and PD was established in 1997, when a point mutation (A53T)[1] in the αS gene was identified in families with autosomal dominant PD [20]. This observation was followed by the seminal discovery of αS as the major component of LBs in brain tissues of sporadic PD cases [21] and the positive immunostaining of these LBs with anti-NACP antibodies [22]. The following year, it was shown that αS was present in LBs as 5–10 nm thick fibrils. In these fibrils αS monomers organized in β-strands that are oriented parallel to the fibrillar axis [23]. These discoveries triggered tremendous scientific interest in αS and the possible causality of αS aggregation and the development of PD. The following year, reports appeared showing that diseased related familial mutants of αS accelerated fibril formation. The accelerated formation of fibrils suggested a direct link between aggregation of αS and the development of early onset PD [24]. Triplication of the αS gene was also shown to cause early onset PD [25] and mRNA levels of αS were found to be consistently elevated in brains of both early onset familial PD [26] and idiopathic PD patients [27]. αS amyloid deposits were also detected in several other neurodegenerative diseases, including multiple system atrophy (MSA), amyotrophic lateral sclerosis (ALS), dementia with Lewy bodies (DLB) and Hallervorden-Spatz syndrome. These diseases are now collectively referred to as synucleinopathies [28]. It is worthwhile to note that often neurodegenerative disorders consist of a continuum of amyloid-related proteinopathies *i.e.* besides amyloid deposits of αS other amyloidogenic proteins also accumulate amyloid aggregates [29].

1.2. Alpha-synuclein

The synuclein family consists of α-synuclein, β-synuclein, and γ-synuclein. These small highly conserved proteins consist of an amino-terminal domain with a variable number of 11-residue repeats, range from 127 to 140 amino acids in length and are 55–62% identical in sequence. Full length monomericαS is a 140 amino-acid protein. Its sequence can be divided into three major regions: the N-terminal region, the NAC region and the C-terminal region. The N-terminal region comprises of amino acid residues 1–60 and can organize into a membrane binding amphipathic helix. The hydrophobic NAC region comprises residues 61–94 and can organize into cross β-sheets. The NAC region is therefore required for aggregation into amyloid fibrils. Part of the NAC region is also involved in membrane binding and seems to define the affinity of αS for lipid membranes [30]. The negatively charged C-terminal region comprises residues 95–140 and is highly unstructured and weakly hydrophobic. It experiences weak and transient interactions, if any, with model lipid membranes [30] (Fig. 2). The C-terminal region remains unstructured in the amyloid fibril and truncations of this region have been shown to modulate aggregation of αS into amyloids [31–33]. The C-terminal region contains sites that can be post-translationally modified by *e.g.* nitration and phosphorylation [34–36]. Whether these modifications are related to function or result in functional disorders is not known. It is however clear that they affect the net charge of the C-terminal region and have profound impact on the αS aggregation and membrane binding as discussed in the later section. β- and γ-synucleins differ from α-synuclein by virtue of the deletion of 11 amino acids in the NAC region and a shorter C-terminal domain respectively. The β- and γ-synuclein proteins are not found in LBs, but both are associated with hippocampal axon pathology in Parkinson's disease and dementia with LBs [37].

Till today, 5 additional point mutations, besides the aforementioned A53T, in the αS gene have been identified that lead to protein variants found in familial forms of PD:A30P [38], E46K [39], A53E [40], H50Q [41] and G51D [42]. Interestingly, all known disease-linked point mutations reside in the membrane binding N-terminal region of αS suggesting that changes in the membrane interactions of monomeric αS are relevant to PD. Although there seems a clear link between mutations in αS and the onset of PD, the role of αS in the disease etiology is constantly debated and finding the mechanism(s) responsible for cellular damage in PD remains a holy grail.

In PD, αS assembles into oligomeric aggregates and amyloid fibrils. Oligomeric protein aggregates have been suggested to play a pivotal role in PD and are often referred to as the more toxic aggregate species responsible for cell death [43–46].Oligomeric αS was first observed in *in vitro* studies on the aggregation of recombinantly produced αS [24]. Subsequently, αS oligomers were reported to be present in postmortem brain of patients with PD [47], cell line cultures [48–50] and neurons [51]. High oligomer concentrations have been associated with disease, but the toxic mechanism responsible for oligomer induced cell death is still under debate [47,52]. The observation that different types of oligomers exist and that they possibly each contribute differently to toxicity in PD complicates this debate [53]. The best characterized αS oligomer species in *in vitro* studies appears in simple buffer solution without aggregation stimulating additives such as dopamine metabolites, fatty acids, or heavy metal cations (reviewed in [54]). This oligomer consists of approximately 30 monomers [55–57]. The oligomer contains some β-sheet structure [58–60], and the C-terminal region of the protein remains flexible and solvent exposed [60,61]. The β-sheet structure of oligomeric αS species has been reported to be distinct from that of fibrils; oligomers were observed to contain antiparallel β-sheets whereas in αS fibrils monomers are typically organized parallel β-sheets [62]. The N-terminal region of the protein in the oligomers retains some of its membrane binding properties [63]. For many of the proposed toxic mechanisms that involve oligomers, the ability to interact with membranes seems to be essential. These mechanisms include membrane thinning [43], pore formation [46], enhanced lipid flip-flop [64] and vesicle clustering [65].

High resolution structures of αS fibrils have been obtained from micro electron diffraction (microED) studies on αS segments [66], solid-state NMR studies [67–69] and more recently the atomic structure of the N-terminally acetylated αS full length and a truncation variant comprising of residues 1–121 was determined from Cryo-EM images [70,71]. However, whether the Greek-key topology reported in the aforementioned studies, is also adopted by αS in *in vivo* formed amyloid fibrils remains to be investigated. Structural characterization of αS amyloid fibrils that are formed *in vivo* or *in vitro* is complicated by the existence of different fibril polymorphs and the sensitivity of the fibril structure to the aggregation conditions used [72,73]. Structural polymorphs of αS fibrils have been shown to result in significantly different toxicities in neuronal cell cultures [68,74]. A recent study has shown that αS fibrils can be internalized, bind plasma membranes of both neuroblastoma cell lines and hippocampal primary neurons and induce cell death when besides fibrils αS monomers are present [75]. The proposed mechanisms of amyloid fibril-mediated membrane damage include membrane deformations and lipid extraction [76] and the activation of apoptotic cell-death pathways [75].

[1] A point mutation in the human αS gene leading to production of a αS variant in which the amino acid alanine at position 53 is substituted by threonine due to a single nucleotide substitution.

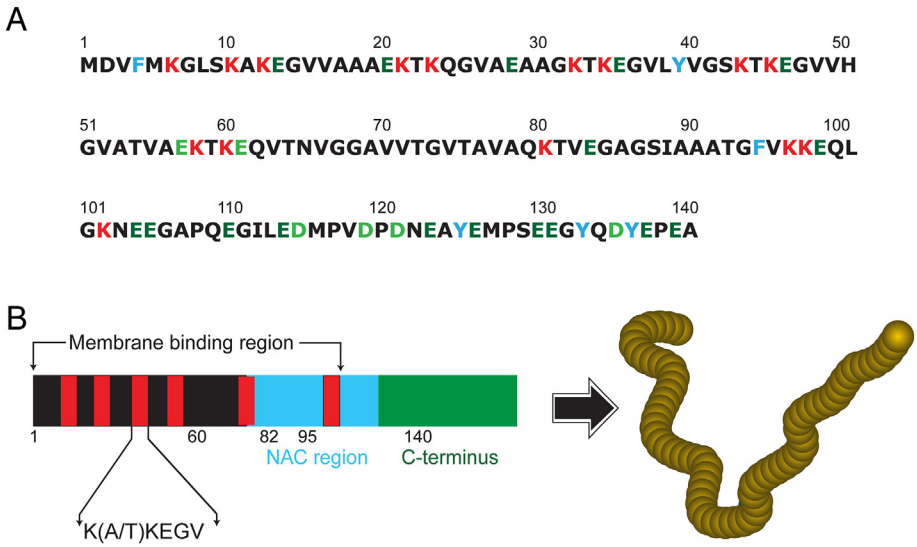

Fig. 2. Sequence encoded physicochemical properties of **αS**. A) Primary amino acid sequence with acidic (green), lysine (red) and aromatic (light blue) residues highlighted. B) Schematic representation of the main (functional) regions of **αS**. The amphipathic repeats housed in the membrane binding region are indicated in red, the Non-Amyloid β Component (NAC) region in blue and the acidic region in green. The right panel shows a pictorial representation of a disordered conformation of the protein.

Not only the role of αS in PD but also the physiological function of αS still eludes us. However, several decades of research have resulted in important progress towards understanding its function as enumerated in the next section. It is now known that αS is predominantly found in the presynaptic terminals of neurons in the human brain. Lower amounts of the protein are present in other tissues. In these cells and tissues, the N-terminal methionine of αS is post-translationally modified; αS is acetylated in its physiological monomeric state [77–79]. Although αS was reported to exist as a stable α-helical tetramer [77,80] reports from other labs failed to confirm the existence of a tetrameric αS species [78,81]. The monomeric N-terminal acetylated form is therefore generally believed to represent the functional form of the protein.

αS has been reported to have many interaction partners including cellular membranes and an enormous number of different proteins [82]. This led to the suggestion that αS may act as an interaction hub for different binding partners [83].Although we focus on (toxic) membrane interactions in this review, the whole picture is probably more complicated since interactions in such a putative hub are interdependent *e.g.* the depletion of αS monomer pool by aggregation may disrupt distribution over functional interactions. Membrane bound αS has been suggested to play a role in regulating synaptic vesicle pools [84], vesicle trafficking [85,86] and vesicle fusion events at the synapse [87]. The mechanism by which αS regulates these processes may depend on physical membrane properties. The N-terminal acetylation of the protein does not seem to be important for αS's interaction with artificial lipid membranes but maybe crucial for aggregation into amyloid fibrils [88] or be relevant for other binding partners [88].

2. αS and phospholipid membrane interactions

αS binds model phospholipid bilayers with equilibrium dissociation constants (K_d) in the micromolar range [89]. This relatively low affinity of αS to model membranes may seem counter-intuitive as a large fraction of the membrane binding N-terminal region of αS consists of imperfect 11-amino acid residue repeats that resemble those found in strongly membrane binding apolipoproteins. These repeats contain a K(A/T)TKEGV consensus that is consistent with the capacity to fold into an amphipathic helix [90] with a periodicity of 11 amino acids per 3 turns [91,92]. However, in contrast to what is observed for αS, the conformational energy landscape of apolipoproteins is characterized by a well-defined energy minimum that represents the folded state. The conformational energy landscape of IDPs such as αS is much more continuous, this means that αS loses conformational entropy upon binding. This loss of entropy decreases the free energy gain upon binding and thus increases K_d. In brain tissue and cell model systems, it is proposed that the relatively low affinity of αS for membranes allows for regulation and control over the distribution between the membrane bound and unbound form of the protein [93,94]. The possibility to reverse binding may for instance be important in regulating the lipid vesicle pool at the synapse [84,95,96]. Förster resonance energy transfer (FRET) studies indicate that there is a clear difference in conformation between cytosolic and membrane bound αS [97]. From experiments on brain homogenates, it was estimated that approximately 15% of αS in brain is associated with membranes [98,99]. This membrane associated αS is visible as distinct high-intensity puncta in fluorescence microscopy images [97]. The high intensity puncta represent αS bound to small vesicles. Photobleaching studies on αS-GFP expressing differentiated SH-SY5Y cells show that approximately 70 αS molecules are associated with each vesicle [100]. The number of αS per vesicle is high enough for a direct role in curvature generation and membrane remodeling [101,102]. However, this number is also strikingly similar to the number of synaptobrevins, a putative interaction partner of αS, per vesicle [103]. This suggests that membrane bound αS has a dual role, it may both directly contribute to generating curvature and act as a non-classical chaperone for SNARE-complex assembly [87].

Association of the unstructured monomeric αS with model phospholipid membranes is accompanied by a dramatic increase in the helical content of the protein from 3% to ~80% [90]. In a seminal report by Eliezer and colleagues in 2001, membrane bound-αS was shown to assume a bipartite structure with residues 1–102 bound to SDS micelles while the remaining residues stay disordered [104]. The amphipathic helix of membrane bound αS is oriented parallel to the membrane surface. The conformation of the membrane-bound helical segment of αS has been a matter of debate as to whether it is a fully extended helix [91,105,106], a broken helix [107,108] or co-existence of both as shown *in vitro* [109] and *in vivo* [97]. It seems that all these helical architectures are possible, probably due to variable positions of the break in the helix [105]. The distribution of αS over the extended and broken helix conformations may depend on the lipid composition of the membrane. Besides the lipid composition, the surface concentration of the protein seems to have an effect on the conformation. It has been shown by NMR that αS binds to lipid bilayers *via* distinct binding modes [110] that can be tuned by changing the lipid-to-protein ratio. Nucleation of αS aggregation may be assisted by membranes that bind the monomeric form of the protein. The critical aggregation concentration of αS in solution has been reported to be ~10–30 μM [111,112]. In the presence of negatively charged lipid bilayers aggregation is however

observed at much lower concentrations, far below the concentrations reported for αS in cells [113–115].

2.1. Physicochemical properties of lipids aiding αS membrane interaction

There is now strong evidence that the equilibrium dissociation constant and thus the population of the membrane-bound state of αS is not only affected by the sequence encoded (structural) properties of αS but also by the physicochemical properties of the phospholipid bilayer, such as anionic charge density, curvature and packing defects, phase state and degree of hydration [90,107,116–118] [119]. Below we will address how the binding of αS monomers, oligomers and fibrils is affected by physicochemical membrane properties. In reviewing the interaction of oligomers with lipid bilayers we will focus on oligomers prepared in simple buffer solutions in the absence of additives. For these types of oligomers, the relation between oligomer interactions and the physicochemical membrane properties of membranes is relatively well characterized.

2.1.1. Membrane charge

2.1.1.1. Monomers. Although net negatively charged, monomeric αS binds membranes of anionic phospholipids with much higher affinity than membranes composed of zwitterionic ones [89,120]. The preferential binding of αS to negatively charged membranes in comparison to net neutral membranes is attributed to attractive electrostatic interactions between the membrane surface and multiple positively charged lysine residues found in the N-terminal region of αS [121]. In the membrane bound conformation, these lysines are lined up at the boundary between the hydrophobic and hydrophilic part of the amphipathic helix. The higher affinity for negatively charged membranes thus likely originates from the free energy gain resulting from both helix insertion and attractive electrostatic interactions. The involvement of attractive electrostatic interactions between αS and negatively charged membranes is corroborated by studies showing reduced αS binding to anionic lipid vesicles with increasing ionic strength [122]. The increased ionic strength not only screens the membrane charge but also changes the conformational space probed by the protein. The changes in preferred protein conformations with ionic strength are a possible cause for the reduced binding affinity. The binding of monomers seems to be very sensitive to the membrane surface charge density. The binding of αS to bilayers of phosphatidic acid (PA) and phosphatidylinositol(PI)from bovine liver lipids that have a slightly higher negative charge at neutral pH, is high compared to binding to bilayers of phosphatidylserine (PS) and phosphatidylglycerol (PG) [89,118,122,123]. For membranes composed of mixtures of zwitterionic- and anionic phospholipid the affinity for αS decreases with increasing fraction of zwitterionic lipids [89,120].

2.1.1.2. Oligomers. Like monomers, the binding of αS oligomers to membranes depends strongly on the presence of negatively charged lipids. The accumulated data from several experiments indicates that for binding to occur to giant unilamellar vesicle (GUV) or large unilamellar vesicle (LUV) membranes at least 20% of the lipids in the membrane must carry a net negative charge [58,124,125]. The ability of the negatively charged oligomers to specifically bind net negatively charged lipid bilayers suggests that, like observed for monomers, oligomer binding is mediated by the lysine residues that reside at the membrane interface when the N-terminal region of the protein is organized into an amphipathic α-helix. The organization of the N-terminal residues of (part of the) monomers in the oligomers into membrane bound amphipathic helices is supported by several experimental findings. In the presence of small unilamellar vesicles (SUVs), the helical content of αS oligomers has been observed to increase [126,127]. Tryptophan fluorescence experiments on single tryptophan mutants of αS indicate that upon membrane binding the environment experienced by residues in the N-terminal region of the protein becomes more hydrophobic [61]. Studies on N-terminal deletion mutants also point at the importance of the N-terminal region of αS for the binding of αS oligomers to membranes [63]. Membrane leakage experiments indicate that, as expected for the binding of negatively charged oligomers to negatively charged membranes, oligomer binding is facilitated at higher ionic strengths [59].

2.1.1.3. Fibrils. Part of the membrane binding N-terminal region of αS remains unstructured and solvent exposed when the protein is organized in amyloid fibrils. Both *in vitro* and *in vivo* experiments suggest that this N-terminal region of αS might retain its membrane binding properties in the fibril state [59,128,129]. Solid-state nuclear magnetic resonance (ssNMR) studies on αS fibrils formed in the presence of negatively charged phospholipid vesicles show that the overall fold of the αS amyloid fibril is not affected by the presence of anionic phospholipid vesicles. However, there are major structural differences between the N-terminal domains of αS in fibrils formed in the absence or presence of vesicles [130,131]. The ability of fibrils to bind vesicle membranes may however depend on the assembly mechanism and the associated differences in fibril polymorphs that are formed. The interaction of pre-formed fibrils with phospholipid vesicles seems to differ from the interaction of fibrils that were assembled in the presence of liposomes.*In vitro* studies indicate that pre-formed αS amyloid fibrils do not bind vesicles of the zwitterionic lipid POPC and show only weak adherence to negatively charged vesicles (50% POPG/50% POPC). However aggregation of αS in the presence of the same POPG/POPC vesicles results in fibrils that strongly adhere to the membrane and deform GUVs [76]. Besides deforming vesicles, the fibrils growing on the anionic lipid bilayer surface can also extract lipids from the bilayer resulting in protein/lipid coaggregates [76,129,132].The lipid composition dependent interactions between αS fibrils and membranes is reflected in the membrane damage caused by fibrils that appear at the membrane surface. Calcein leakage measurements show that the growth of αS fibrils in the presence of vesicles does not impair the integrity of phospholipid vesicles that have a low affinity for αS [132], while fibril growth does cause dye leakage with increasing fraction of anionic lipids in membrane [59,133]. A recent study has shown that αS fibrils can bind plasma membranes of both neuroblastoma cell lines and hippocampal primary neurons and induce cell death when αS monomers are additionally present [134].

2.1.2. Membrane curvature

2.1.2.1. Monomers. Membrane binding of αS involves a conformational transition from a disordered state to an amphipathic α-helix and the insertion of this helix into the lipid bilayer. Binding of αS to membranes therefore depends on membrane tension and the presence of packing defects [90]. The sensitivity to the presence of packing defects is probably responsible for the curvature sensitivity of αS binding. αS has been observed to bind SUVs with much higher affinity than larger vesicles. Moreover, whereas αS does not bind LUVs and GUVs of zwitterionic lipids it does bind SUVs of zwitterionic phospholipids [88]. In SUVs with a diameter of 25–40 nm the vesicle diameter starts to approach the lipid bilayer thickness which results in an imperfect packing of lipids and the formation of membrane defects. These packing defects become even more pronounced when the membrane of these SUVs is in the liquid-ordered or gel phase.The exposed hydrophobic surface at defects is probably responsible for the observed higher binding affinity of SUVs compared to LUVs and GUVs.Similarly, increasing the fraction of inverted cone-shaped lipids with packing parameter[2] $P > 1$, like phosphatidylethanolamine (PE) in anionic lipid vesicles enhances binding of αS [59,122].

[2] Packing parameter, P = V/a*l, where V is the hydrocarbon volume, a is the area of the head group, and l is the length of the hydrocarbon chain)

Interestingly, αS not only binds preferentially to curved lipid membranes but has also been shown to induce curvature and cause remodeling of lipid membranes [102,135]. The binding of αS has been shown to convert multilamellar vesicles and GUVs but not SUVs of physiologically relevant phospholipid compositions into tubules and smaller vesicles [101]. Separate studies showed that membrane remodeling of supported lipid bilayers by αS strongly decreases with increasing anionic lipid content [136]. The membrane remodeling effect observed in these studies is likely a result of the partial insertion of an amphipathic helix in the outer layer of the membrane [101], although the steric pressure exerted by the solvent exposed C-terminal regions of the protein may also contribute at high surface densities [100,137,138]. Considering the weak binding of monomeric αS the observed increase in the tubulation of zwitterionic supported lipid bilayers is puzzling [136].

2.1.2.2. Oligomers. Although the binding of αS oligomers to SUV, LUV and GUV membranes was not directly compared in a single series, binding seems to depend on curvature. Oligomers have been reported to bind DOPC SUVs but not POPC GUVs [124,139]. This may however not result from the ability of oligomers to sense curvature. The inability of αS oligomers to bind POPE SUVs [124,139] suggests that binding to SUVs of zwitterionic lipid bilayers results from packing defects and depends on the exposure of hydrophobic surface. In some, but not all cases the binding of oligomers results in impaired membrane integrity. Whereas αS oligomer binding requires the presence of negatively charged phospholipids, disruption of the lipid bilayer depends on the accessibility of the bilayer hydrocarbon core [59]. This accessibility of the hydrocarbon core cannot only be modulated by changing the membrane curvature and creating packing defects, it also depends on the packing parameters of the lipids in the bilayer.In a series with increasing acyl chain unsaturation; POPG, DOPG, 18:2 PG, the packing parameter P increases and lower oligomer concentrations are required for calcein to leak out of vesicles composed of these lipids. The addition of the more cone shaped ($P < 1$) lysolipids improves the packing of lipids in the bilayer and decreases the vulnerability of the membrane to oligomer binding induced impairment of membrane integrity [59]. The addition of cholesterol also improves the packing of the lipid bilayer and has a similar effect [59,139]. In general, it has been observed that although lipid bilayers of more complex physiologically relevant membrane compositions bind oligomers, such membranes are less vulnerable to binding induced leakage of vesicle content. The dye release caused by αS oligomer binding from vesicles of brain extract [139,140], or vesicles mimicking the composition of the plasma membrane or mitochondrial membrane [127], is low compared to the release from vesicles containing only negatively charged lipids. There are however differences, SUVs mimicking the composition of the inner mitochondrial membrane were more susceptible to permeabilization by αS oligomers than model plasma membranes [127,141].Whereas the main acidic phospholipid in the plasma membrane is the phosphatidylserine, the inner mitochondrial membrane is enriched in cardiolipin; a diphosphatidylglycerol carrying two negative charges. The sensitivity of oligomer binding to membrane composition and charge density may make the membranes of specific organelles more vulnerable to oligomer induced damage than others [142,143].

2.1.2.3. Fibrils. To the best of our knowledge, membrane curvature dependence of αS fibril binding has not been investigated yet. However it has been shown that when monomeric αS is aggregatedin presence of membranes, membrane bound fibrils can deform both SUVs [129] andGUVs [76].

2.1.3. Membrane phase state

2.1.3.1. Monomers. Studies on permeabilized cells from rat brains suggest that αS is associated with lipid rafts in the cellular membrane [144]. These lipid rafts are defined by their resistance against treatment with anionic detergent and are typically enriched in cholesterol, sphingolipids and specific phospholipids. In membrane model systems, these rafts are often mimicked using binary or tertiary lipid mixtures containing phase separated liquid ordered (L_o) domains. In contrast to what has been observed in cellular membranes, αS typically binds the anionic lipids in the liquid disordered (L_d) instead of the L_o phase in these phase-separated systems [120]. However, it is important to keep in mind that there is no direct evidence that the lipid rafts in cells are L_o phases. Cellular membranes are not equilibrium structures and raft formation may result from various internal driving forces rather than from phase separation as discussed in [145].

The affinity for different membrane phase states has been studied in more detail in simpler membrane model systems. The phase state of membranes *e.g.* L_o, L_d, or gel state, depends on lipid composition and temperature. For anionic membranes, the binding affinity of αS is higher for SUV membranes with lipids in the fluid phase than for membranes in the gel phase [146]. For SUVs and LUVs composed of zwitterionic lipids the effect is reversed, αS dissociates from these vesicles upon the phase transition to the L_o state [89]. The phase transition temperature and therefore binding of αS depends strongly on the length of and degree of saturation in the acyl chain of the lipids. However, also above the L_o to L_d transition temperature the binding of αS is affected by the degree of saturation in the acyl chains of the lipids in the bilayer. Compared to membranes composed of saturated lipids, binding of αS to membranes of unsaturated lipids of the same length is higher [89,120]. The sensitivity of αS binding to the membrane composition and phase state of membranes of zwitterionic lipids indicates that exposed hydrophobic area enhances binding. Defects in membranes below the phase transition temperature and the less tight packing of unsaturated compared to saturated lipids in membranes above the phase transition temperature reduce the screening of the apolar acyl chains in the bilayer. Membrane defects and decreased lipid packing thus both enhance the insertion of the amphipathic helix of αS into the bilayer.

It is important to realize that the phase state and lipid packing not only affect binding of αS but that binding also changes these parameters [107,116,147]. EPR and fluorescence spectroscopy show that binding of αS to SUVs of zwitterionic lipids led to an increased chain melting temperatures and to enhanced cooperativity of the phase transition [148]. By contrast, CD and DSC results suggest that αS binding stabilizes the fluid phase of bilayers of negatively charged lipids. Experiments from our own lab indicate that αS organizes in clusters at protein:lipid ratios higher than 10 [113]. At such higher ratios, all αS binding sites on the membrane surface are occupied, the average distance between two membrane-bound monomers is small and interprotein collisions result in cluster formation. Clustering of membrane-bound αS at high surface concentration is a consequence of a complex interplay mainly between attractive hydrophobic interactions, resulting from the solvent exposed hydrophobic patches on the membrane-bound αS, and repulsive electrostatic interactions, resulting from the negatively charged unstructured solvent exposed C-terminal region of αS. The correlation between the changes in lipid diffusion and DPH anisotropy, suggests a concerted process where the formation of clusters leads to a closer packing of lipids and a decrease of the effective lipid diffusion [149]. The relation between the effective lipid diffusion and area of the αS clusters (Fig. 3) is nontrivial as it does not result from a direct interaction between proteins and lipids. Although aS mainly binds to anionic lipids, clustering also affects effective lipid diffusion of zwitterionic lipids. This indicates that the clustering of αS on lipid membranes induces ordering of underlying lipids. In the L_d membranes studied, we observed an overall increased lipid packing order with increasing size of the lipid clusters (Fig. 3).

For membranes in the L_o state, the opposite has been reported; αS decreases the packing order in lipid mixtures that form L_o membranes [51]. Cholesterol affects the packing order in lipid membranes in a similar way; it increases the packing of membranes in the L_d state and

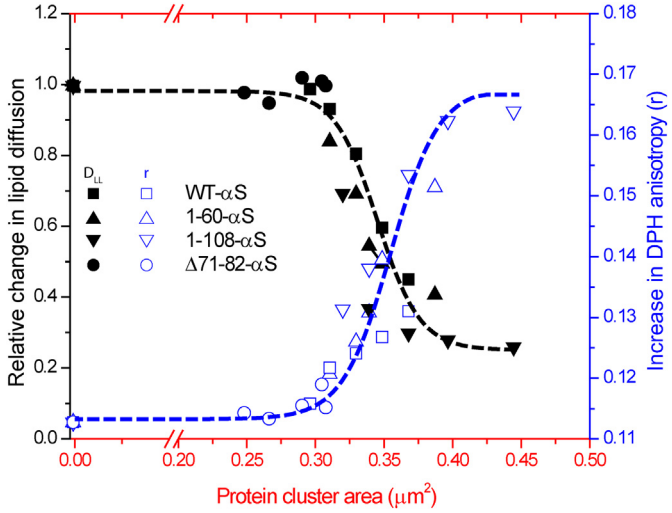

Fig. 3. Master curve of data correlating changes in lipid diffusion (black solid symbols) and lipid packing (blue open symbols) measured as the absolute steady-state anisotropy values of DPH in liposomes to membrane-bound αS cluster areas. Relative changes in the lipid diffusion coefficients are plotted against mean αS cluster areas. WT-αS is depicted as squares whereas the Δ71–82-αS variant is shown as circles. The 1–108-αS variant (downward triangles) results in the biggest change in lipid diffusion coefficients and DPH anisotropy followed by the 1–60-αS variant (upward triangles). The dotted lines are representative of the general trend in increasing anisotropy (blue lines) and changes in lipid diffusion (black lines). (Figure and legend reused with permission from [149].)

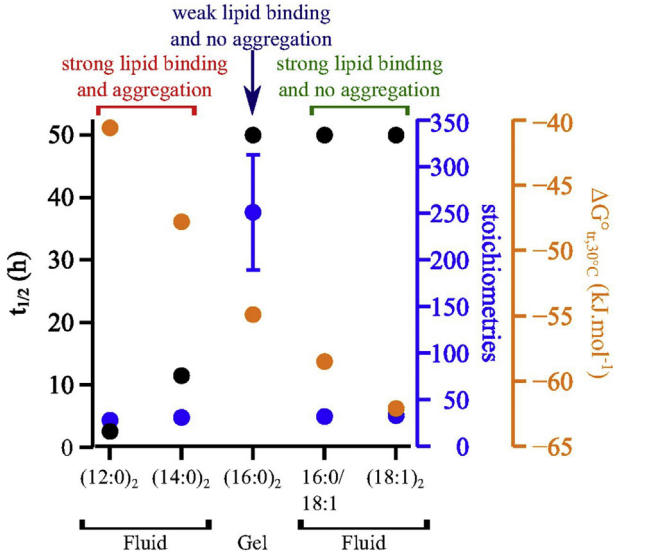

Fig. 4. Physicochemical properties of lipids influence the binding stoichiometry and the aggregation propensity of αS in the presence of model membranes. The energy gained by transferring a lipid molecule from the water phase into the bilayer (ΔG°_{tr}, 30 °C) (orange), the stoichiometry of αS:lipid binding in the bound state (blue), and the half-time for the aggregation of αS (black) are plotted for each lipid system: DLPS [(12:0)2], DMPS [(14:0)2], DPPS [(16:0)2], POPS (16:0/18:1), and DOPS [(18:1)2]. The phase of each lipid systems in the presence of an excess of protein at 30 °C is indicated below the x axis. (Figure reused with permission from [146].) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

makes membranes in the L_o state more fluid. Clustering of proteins and ordering of lipids into membrane microdomains are both known to be involved in protein function and this interplay forms the basis for many cellular signaling processes [145]. The aforementioned studies on αS suggest, albeit from *in vitro* observations, that αS may play a role in the regulation of lipid packing in cell membranes.

2.1.3.2. Oligomers. Preferential binding of αS oligomers to membranes in the L_o over the L_d state was studied in GUV membranes with coexisting L_d and L_o domains. In these experiments oligomers preferentially accumulated in the L_d domains [124]. Additionally, vesicles of anionic lipids in the L_o state were not able to bind oligomers [124]. The close packing of lipids in the L_o state probably interferes with the insertion of the amphipathic helix even when the surface charge is high enough. Considering the sensitivity of αS oligomer binding to packing defects, one could imagine that αS oligomers preferentially accumulate at the interface between the L_o and L_d state. There are however no indications that is the case.

2.1.3.3. Fibrils. To the best of our knowledge, the binding of preformed fibrils to vesicles with membranes in different membrane phase states has not been investigated yet. However, when fibrils were formed in the presence of net negatively charged SUVs or GUVs large scale vesicle deformations were observed [76,129]. Fibrils at the membrane surface cause the initially spherical GUVs to become faceted. This faceting may indicate that the binding of fibrils causes the membrane to become more rigid or to develop rigid domains. However, dye leakage studies and the distribution of dyes which preferentially accumulate in L_d over L_o domains indicated that the tight coupling to a mesh work of fibrils, rather than a rigidification of the membrane, is the more likely cause of the observed vesicle shape changes [76].

The aggregation of αS on or in the presence of membranes seems to be affected by the membrane composition. Despite the fact that monomeric αS binds with similar affinities to model membranes composed of different negatively charged phospholipids, it was shown recently that the aggregation of the protein is enhanced only in the presence of membranes composed of lipids with the shortest hydrocarbon chains (Fig. 4) and hence highest solubility [146,150]. Membranes composed of phospholipids with 16 or 18 carbons in each acyl chain including DOPE, DOPC, DOPS, POPS do bind αS but do not enhance the aggregation of the protein when incubated for several days under quiescent conditions [146]. Experiments in which αS aggregation was followed in the presence of LUVs composed of POPG:POPC (1:1), POPG:POPE:POPI (11:3:6), or POPC/POPE/Cardiolipin (5:3:4) also showed no enhanced αS aggregation [132]. The standard change in free energy of transfer of a lipid molecule from water into a bilayer, and thus the solubility, correlates strongly with the length of its lipid hydrocarbon chain(s) [151] (Fig. 4). Lipids with the highest solubility in aqueous solutions triggered αS aggregation. This is in good agreement with earlier experiments were αS aggregation was followed in the presence of SDS below and above the critical micelle concentration [152]. Below the CMC, the fibril formation process was concluded to be mediated by the formation of micelle like clusters at the surface of αS molecules. Above the CMC, the presence of SDS micelles inhibited fibril formation, probably because the presence of micelles decreases the concentration of free/unbound αS in solution. In summary, the high solubility of single chain surfactants and (charged) phospholipids with short acyl chains results in a considerable concentration of these amphiphiles in solution. These solubilized lipids and surfactants interact with αS and induce its aggregation. The ability of vesicles to increase the aggregation rate and decrease the aggregation lag time thus depends on lipid solubility rather than membrane phase state.

Although αS aggregation is not accelerated in the presence of vesicles of less soluble phospholipids, the low solubility of these lipids cannot prevent lipid extraction when membrane bound αS is incorporated in fibrils [132]. Aggregation of membrane bound αS has been reported to result in extraction of lipids and the appearance of protein/lipid co-aggregates [76,115,129,132,153]. This lipid extraction can consume the complete vesicle bilayer and is therefore disruptive.

In summary, it seems that the interactions of αS monomers, oligomers and fibrils with phospholipid membranes respond similarly to the membrane's physicochemical properties. Besides the physicochemical properties of membranes discussed above, interactions between αS and membranes are also influenced by the chemical composition of the membranes and the presence of specific lipids. How the chemical composition of membranes affects the aggregation of αS has recently been reviewed by [153] and will not be addressed here.

Most of the investigations on the relation between the physicochemical properties of membranes and the interactions with different αS species have been performed on the non-acetylated version of αS while *in vivo* most αS contain this post-translational modification. Do monomeric, oligomeric and fibrillar N-terminally acetylated αS species respond differently to changes in membrane properties? Will N-terminal acetylation of αS proteins containing one of the familial PD substitutions change our view on αS-membrane interactions and αS mediated pathology in PD? Addressing these questions may bring us a step closer to understanding the role of αS-membrane interactions in PD.

2.2. Post-translational modifications and mutations in αS influencing membrane interactions

In vivo, both the monomeric and fibrillar form of αS have been observed to be post-translationally modified. The reported post-translational modifications (PTMs) include acetylation [34,79,88,154], phosphorylation [34,155–157], methionine oxidation [158], nitration [159], ubiquitination [34,160], SUMOylation [161] and truncations. The relevance of PTMs for αS function and the role of PTMs in the etiology of PD are not clear yet. Considering that several of the PTMs affect the net charge or charge distribution on the protein, PTMs change the aggregation propensity and may influence the distribution of the monomeric protein over (functional) conformational sub-ensembles. Post-translational modifications may thus directly or indirectly impact αS interactions with lipid membranes. PTMs can thus either have a regulative effect on cell physiology for functional purposes or be disruptive to the existing function of αS leading to PD pathology. The consequences of these PTMs for the interaction of αS with membranes will be discussed in this section.

2.2.1. N-terminal acetylation

The main functional PTM found in human αS is N-terminal acetylation [34,79,154,162,164]. How this acetylation affects the αS membrane binding ability has been under debate. Initially conflicting results were reported; N-terminal acetylation was observed to enhance membrane binding in some studies while a negligible impact of the PTM was reported by others [154,162]. Recent investigations have shown that N-terminal acetylation enhances binding to SUVs containing no or a low percentage of negatively charged phospholipids. For membranes with higher surface charge densities the effect of the PTM on binding was much less pronounced [88,163]. Considering the importance of the positively charged amino acid residues in the N-terminal region of αS for binding to anionic phospholipid bilayers the loss of a positive charge upon acetylation is expected to decrease the affinity for anionic lipid bilayers. However, N-terminal acetylation increases the propensity of the first 14 residues of the protein to organize into helices. The helical content of the N-terminally acetylated protein in buffer is considerably higher than that of the unmodified protein [162]. Thus, the loss in conformational entropy upon binding to anionic phospholipid membranes is probably lower for N-terminally acetylated αS than that for the unstructured unmodified αS. The loss of the positively N-terminal residue upon acetylation is balanced by the lower entropy cost associated with helix formation. Binding of unmodified and N-terminally acetylated αS to negatively charged lipid bilayers is therefore comparable. Since binding of αS to bilayers of zwitterionic lipids does not strongly rely on attraction between oppositely charged surfaces, the effect of N-terminal acetylation is dominated by the increased propensity of the protein to fold into an amphipathic helix.Because the final helical content of both membrane-bound proteins is comparable, the net free energy gain upon binding of unmodified αS to membranes of zwitterionic lipids is lower resulting in a measurable increase in affinity for N-terminally acetylated αS compared to unmodified αS [88].

2.2.2. Phosphorylation

Whereas N-terminal acetylation is functional, phosphorylation of αS is associated with disease conditions. According to early reports approximately 90% of αS in LBs is phosphorylated at Ser129 (pS129) [164] whereas only a small proportion (< 5%) of the monomeric protein contained this PTM. Later studies indicated that besides pS129, phosphorylated Ser87 can be considered a pathological hallmark of αS inclusions [165]. Based on the presence of phosphorylated protein in LBs, it was hypothesized that phosphorylation of αS promotes aggregation *in vivo*. Subsequent *in vitro* studies confirmed this; phosphorylation of αS at residues S87 and Ser129 was observed to induce the formation of relatively extended conformations exposing the aggregation prone NAC region and increasing the aggregation propensity [165–168]. However, other *in vitro* aggregation assays could not confirm the hypothesis that phosphorylation promotes aggregation [169].

Phosphorylation of S87 reduces the binding affinity to lipid membranes [155,170] and alters the detergent micelle bound conformation [165]. *In vitro* experiments probing the binding affinity of pS129 to phospholipid membranes have, like the aggregation studies, not been able to generate a unified view. Some studies report that phosphorylation at S129 does not change the affinity of αS for membranes and that it only moderately enhances binding of PD-linked mutations of αS (A53T and A30P) [35,164]. Others report a reduction of the membrane binding affinity for αS phosphorylated by a G protein-coupled receptor kinase (GRK) [171,172]. The apparent discrepancy in the effect of phosphorylation on membrane binding could be a result of different kinases used in these experiments; creatine kinase (CK1) and Pollo-like kinase (PLK2) in the first study *versus* GRK in the latter, as reviewed extensively in [169]. These different kinases will most likely differently phosphorylate other residues besides S129. Like observed for GRK phosphorylated αS, the binding affinity of the phosphomimic S129E to membranes was lower than that of the unmodified protein [171,172]. Phosphomimics have generated relatively reproducible data in cellular and animal studies probing the influence of phosphorylation of αS. The αS-membrane interaction was shown to be inhibited by this phosphorylation mimicking αS mutation in yeast and worm models of PD. The mutation of αS to the unphophorylatable S129A variant increases the fraction of membrane-bound αS, while the mutation to the S129D phosphomimic decreases the fraction of membrane-bound αS [173,174]. In an adeno-associated virus (AAV)-based rat genetic model of PD, immuno-electron microscopy images showed that the majority of αS associated with cellular membranes was S129A [175]. However, the phospho-mimics (S129D/E) do not replicate the exact properties of physiologically phosphorylated αS [35,176]. The structural and functional consequences of phosphorylation on lipid membrane binding remain therefore incompletely understood.

2.2.3. Oxidative modifications: tyrosine nitration and methionine oxidation

Their oxygen consumption rate makes dopaminergic neurons quite susceptible to oxidative stress [177]. Under conditions of oxidative stress all four tyrosines in αS (Fig. 5) can be nitrated *in vitro* and this nitration has also been observed in LBs from the brains of PD patients [159,178–180]. *In vitro* nitration of Tyr-39 or the C-terminal tyrosines leads to a decreased binding of αS to membranes composed of anionic lipids [181]. The sensitivity of membrane binding to C-terminal nitration is interesting; the C-terminus is not thought to interact directly with membranes. The sensitivity of αS-membrane interactions to nitration suggests a long-range allosteric communication between the C-

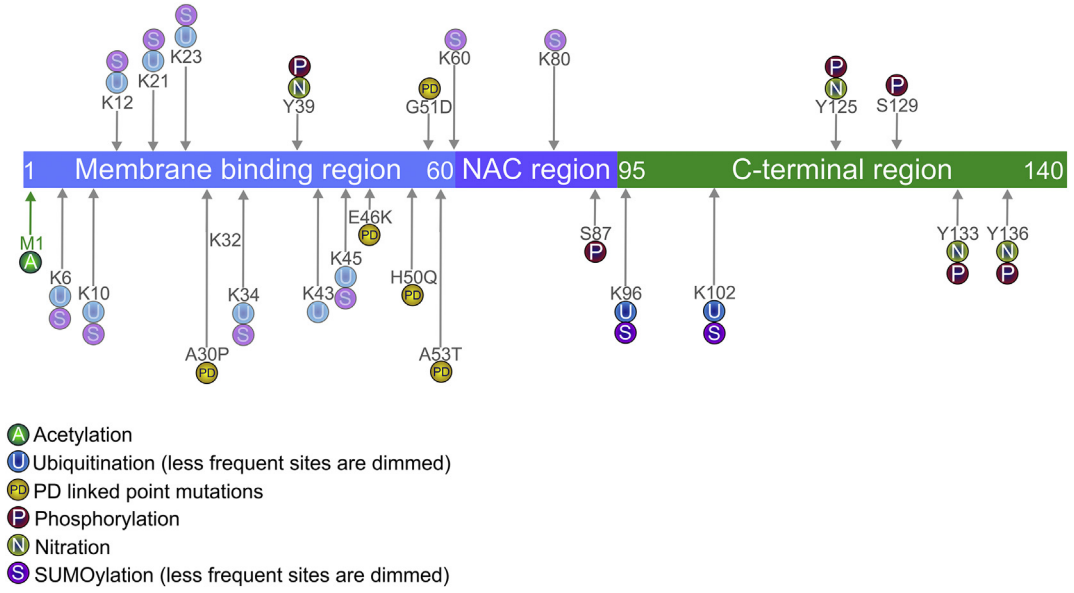

Fig. 5. Overview of post-translational modifications in αS.

terminal and the membrane binding regions. Other oxidative PTMs that possibly influence membrane binding of αS include methionine oxidation [158,182] but the effects of methionine oxidation on membrane binding is not investigated yet.

2.2.3.1. Truncation. Up to 15% of the αS in LBs contains N-terminal and C-terminal truncations [183,184]. *In vitro* experiments have shown truncations can have a large effect on amyloid fibril structure and morphology [185]. However, these PTMs of αS are also commonly found in healthy brain tissue and in cultured cells [186–188].Thus, αS truncation does not *per se* result in disease. The N-terminal region of αS with its imperfect KTKEGV repeat motifs is important for establishing membrane interactions. Truncations of the N-terminal region are therefore expected to negatively affect the membrane binding affinity of αS. The high number of negatively charged amino acids in the C-terminal region may modulate interactions with negatively charged membranes but compared to truncations in the N-terminal region, they are expected to have less effect on membrane binding. Initial studies reported that deletion of the first 10 N-terminal amino acids (residues 2–11) dramatically reduced binding to vesicles and yeast membranes [189]. Concomitant with the lower binding affinity, the overexpression of this deletion mutant was observed to be considerable less toxic than overexpression of the full-length protein. However, although less toxic in yeast, a subsequent study by the same group failed to reproduce the reduced membrane binding and toxicity in human neuroblastoma SHSY-5Y cells [190]. Differences in lipid composition, membrane fluidity and cytosolic factors between yeast and SH-SY5Y cells likely cause this apparent disparity. The observed differences thus indicate that the choice of the model system is critical.

In vitro studies show that the amino acids 1–25 in the N-terminal region of αS trigger membrane binding and helix folding [191]. However, the peptide 1–20 had extremely low affinity for anionic liposomes compared to 1–25 and full-length αS. The low affinity of the 1–20 peptide was attributed to the net zero charge of the peptide. However, experiments with other truncations variants show that a net positive charge is not sufficient for membrane recognition and helix folding. Instead the N-terminal residues impart some kind of conformational selectivity that allows for membrane recognition [191].

More recent studies have confirmed the roles of the N- and C-terminus in membrane binding. Using a small peptide tagged αS (αS-myc), it was shown that truncations in the unstructured C-terminus of αS have minimal effect on lipid binding *in vitro* and do not affect the presynaptic localization of αS in cultured cortical mouse neurons [192]. The C-terminus, however is essential for synaptobrevin-2 binding and promoting SNARE-complex assembly [192]. N-terminal truncations in αS, as expected, decreased membrane binding affinity to artificial lipid membranes and significantly decreased the presynaptic localization of αS in cultured cortical mouse neurons [192]. In general, the behavior of N- and C-terminal truncation variants to membrane binding are comparable *in vitro* and *in vivo*.

2.2.3.2. SUMOylation. SUMOylation is a post-translational modification that involves the enzymatic addition of small ubiquitin-related modifier (SUMO) to proteins [193]. SUMOylation of αS has been shown to occur in HEK293 cells [161] and transgenic mice [194]. SUMOylation is thought to regulate protein-protein and protein-DNA interactions, it additionally promotes protein solubility [195,196]. SUMOylation has been observed on several lysine residues in αS, the most significant of which are lysines 96 and 102. Although the sorting of αS in extracellular vesicles is regulated by SUMOylation and membrane interactions may thus be important it remains to be investigated if *in vivo* or *in vitro* membrane binding of αS is influenced by SUMOylation [197].

2.2.3.3. Point mutations. All known familial PD point mutations resulting in single amino acid substitutions in αS (A30P, A53T, H50Q, G51D and E46K) reside in its membrane binding region (residues 1–60). Compared to unmodified αS, these single amino acid substitutions either promote or impede membrane-associations of αS [110,118,198–200]. Like observed for unmodified αS, the membrane binding affinities of the disease mutants increase with the fraction of negatively charged lipids in the membrane [199,200] or the presence of packing defects in small-sized vesicles [118].

Compared to unmodified αS, A30P shows a reduced binding affinity to artificial lipid membranes. This reduced affinity presumably results from the break in the membrane bound amphipathic helix caused by the presence of a Pro residue [110,118,198,201,202]. In accordance with this hypothesis, mutations of conserved residues in the membrane binding part of αS to prolines resulted in proteins with a reduced membrane binding affinity, with the exception of G41P [192]. For the PD related E46K mutation in αS, an increased affinity for binding phospholipid bilayers is observed which can be attributedto enhanced attractive electrostatic interactions resulting from the presence of an additional Lys residue [110,118,198,201]. There is currently some

inconsistency regarding data reported on the binding affinity of the A53T mutant to artificial lipid membranes. Compared with unmodified αS, A53T showed either reduced [199,203] or similar [110,198,201] binding affinities. The H50Q and G51D mutations have not yet been extensively studied. Compared to unmodified αS, the G51D mutation decreases the membrane binding affinity, but promotes the formation of partly helical states [204], while the H50Q mutation does not alter binding affinity orthe fold of the bound-state significantly [205].

Unmodified αS and disease related mutants bind to artificial lipid membranes using a very similar combination of electrostatic and hydrophobic interactions [118,199,200], but the presence of vesicles differently affects their aggregation into amyloid fibrils. In a study performed in the presence of exosomes derived from mouse neuroblastoma cells the fibrillation rates were observed to decrease in the order A53T > A30P > E46K > unmodified αS [206].

So far, all the PTMs in αS have been investigated separately, studies aimed at probing the combined effects of different coexisting PTMs are still lacking. For example, phosphorylation at Y125, ubiquitination at K96 or K102 or C-terminal truncations co-exist with pS129, although the modification sequence is unknown. Although PTMs in αS occur at multiple sites, certain positions can carry different modifications (Fig. 5). The functional/pathogenic consequences of combinations of PTMs also require further investigations.

3. The Janus face of αS

To explain the role of the conformational transition of αS from its disordered state to the oligomeric and fibrillar states in PD etiology, several mechanisms of αS mediated cellular toxicity and death have been postulated. These mechanisms can be grouped into two major classes: they either assume a gain of toxic function or a toxic loss of function. Both gain and loss of function mechanisms result in failure of the ubiquitin-proteasome system (UPS), oxidative stress, impaired axonal transport and mitochondrial damage [28,98,207–215]. Although not established, gain and loss of function mechanisms may not be mutually exclusive and possibly act synergistically. The interaction between different cellular processes, makes it difficult to pinpoint one single intracellular location or pathway that is affected in the early stages of PD and eventually results in neuronal cell death. Possibly many different pathways contribute to cell death in PD.

In current literature, the roles attributed to αS in PD come in opposites, in this respect the protein seems to resemble the mythological two-faced Roman god Janus. On one hand, in both familial/idiopathic cases of PD [28,74], the failure of cellular processes stems from αS point mutations and the overexpression and aggregation of αS into toxic pre-fibrillar, oligomeric and/or fibrillar species [66,68]. In this respect, soluble oligomeric species of αS have been argued to be the most potent toxic species in both *in vitro* and *in vivo* systems [53,216–219]. However, the observed transmission of the amyloid fold from one cell to another suggests a critical role for fibrils [220]. When particle instead of equivalent monomer concentrations are compared fibrils seem more toxic than oligomers [139].The toxicity of fibrils seems to depend on the fibril strain [74] and the toxicity of LB formations on the cellular compartment in which fibrils accumulate [221].On the other hand, a number of reports suggest that overexpression of αS *per se* and aggregation into soluble oligomeric (formed in presence of dopamine) and fibrillar species has a neuroprotective role in PD [28,213,222–224]. This neuroprotective role of αS is also supported by the fact that PD and the associated death of dopaminergic neurons can also occur without formation of LBs [225,226]. The neuroprotective role of aggregates and the absence of a clear correlation between the number of LBs and disease raises questions about the precise role of αS in PD. The Janus face of αS also becomes visible in the role the protein may play during oxidative stress. The scarcity of defense mechanisms against oxidative damage and a high oxygen consumption rate make dopaminergic neurons susceptible to insult by oxidative stress [177]. Oxidation of brain lipids, polyunsaturated fatty acids (PUFAs) and dopamine in particular, can affect normal functioning of cell membranes. These effects of oxidation increase with age and have been linked to PD [227,228]. Several studies have suggested that αS acts on cellular vesicles acts as an anti-oxidant; αS levels are elevated in neurons exposed to chronic oxidative stress and such neurons showed increased resistance to apoptosis [211]. The binding of monomeric αS to the lipid bilayer prevents oxidation of unsaturated lipids in vesicle models [229].αS has been shown to be protective by interacting with excess dopamine and its oxidized products. In *in vitro*experiments the interaction of αS with dopamine results in the formation of non-toxic oligomeric intermediates [45,127] αSthereby prevents toxic interactions of lipid molecules with excess dopamine species [213,230]. However, the interactions of αS with dopamine are not only beneficial. Increased levels of dopamine have been shown to result in stabilization of protofibrillar species of αS that have been shown to be toxic to cells [231] [232]. αS plays a role in the regulation of dopamine transporters preventing dopamine accumulation in neuronal cells [213]. Deficiencies in dopamine packing into vesicles in mice results in an increase in cytosolic dopamine and a corresponding accumulation of αS which causes death of mice dopaminergic neurons [230,233]. The absence of αS in such mice correlated with better survival suggesting presence of αS aggravates dopamine mediated toxicity [233]. The Janus face of the protein may be a result of its ability to also interact with many other cellular components besides membranes as exemplified by the contrasting findings on its function and cytotoxicity in mitochondria. One of the proposed physiological functions of αS involves the modulation of mitochondrial complex I activity [232,234]. By controlling activity αS prevents mitochondrial complex I induced apoptosis. Additionally, the interaction of αS with apoptosis-promoting proteins promotes resistance against mitochondrial toxins [235]. Lastly, the absence of αS in mitochondria alters the mitochondrial lipid composition and causes a reduction in the activities of complexes I and III [212].However, besides a protective role, the interaction of αS with mitochondrial membranes and membrane proteins in neuronal cells has been reported to be toxic. αS mediated mitochondrial dysfunction due to inhibition of the respiratory chain is thought to be one of the major triggers for both familial and age-dependent PD [236,237]. Inhibition of mitochondrial complexes I and III results in increased oxidative stress and the generation of reactive oxygen species. The inhibition of the oxidative phosphorylation chain and generation of ROS has consequences for active downstream processes including SNARE mediated exocytosis [238]. Other possible mechanisms by which αS binding can result in mitochondrial damage include the release of cytochrome *c*, increase of mitochondrial calcium and concomitant apoptosis [237]. Besides cytotoxic mechanisms that involve oxidative stress, αS aggregates have also been reported to induce fragmentation of mitochondrial membranes [239,240]. This fragmentation of mitochondria is often associated with the degradation of dysfunctional organelles.

4. Conclusions and future perspectives

Although the function of αS and its role in PD remain debated, the interaction of αS species with cellular membranes seem to be important for both function and toxicity. In this respect, the interactions with membranes of intracellular vesicles and mitochondria seem to be most significant.Despite the observations that specific intracellular membranes are targeted by αS aggregation or specific αS aggregates, the molecular mechanisms underlying membrane disruption are in many cases not well understood. The inherent compositional complexity of biological membranes and largely unknown physiological functions of αS impede obtaining molecular insights into the mechanisms by which αS aggregates disrupt cellular membranes. Additionally, the impact of relevant combinations of PTM of αS on membrane binding and aggregation have been largely ignored. In spite of this, the studies on different model phospholipid membranes reviewed here indicate that

the αS residues that enable functional monomer-membrane interactions can become disruptive in αS aggregates. How combinations of structural and binding properties of specific aggregate species confer toxicity is still a largely open question.

Membranes have not only been identified as a direct target of toxic interactions with αS species, interactions of αS with the membrane (lipids) also potentially play a role in the aggregation of the protein. If and how enhanced aggregation results from crowding of protein at the membrane surface and/or is a result of interactions with lipids in solution remains to be investigated. In this respect it will be important to carefully reevaluate both the *in vitro* and *in vivo* membrane model systems of choice. Opposite effects in membrane binding and toxicity have been observed in different organisms. Although differences in interactions of αS with soluble components in these systems will play a role at least part of this difference in membrane binding can probably be contributed to differences in membrane composition. Difference in membrane composition may not only affect the membrane affinity of αS, *in vitro* studies have shown that the lipid composition affects the aggregation pathway. Different pathways have been shown to give rise to oligomeric, fibrillar or amorphous aggregates. All of these aggregates have been implicated in toxicity but they are thought to act *via* different mechanisms. Studies with non-abundant biological lipids or non-physiological membrane compositions may give a wealth of information on interactions and possible toxic mechanisms. However, these mechanisms may have little relevance *in vivo*. Increasing the complexity of model membranes to more closely mimic the properties of cellular membranes may help but a better characterization of the evolution of membrane bound αS species *in vivo* is required.

Taken together, the wealth of data obtained in the last two decades points towards a scenario wherein the interaction of αS with membranes can be neuroprotective but at the same time lipid membranes have been indicated to aid αS aggregation by creating an environment that enhances the assembly of early aggregate species. The interaction of αS aggregates, that form either on cell membranes or in solution, with membranes can result in cellular dysfunction and even cell death.

References

[1] M.J. Schleiden, Beiträge zur phytogenesis, Archiv für Anatomie, Physiologie und Wissenschaftliche Medicin. 13 (1838) 137–176.
[2] R. Virchow, Zur Cellulose-Frage, Virchows Archiv fur pathologische Anatomie und Physiologie (1854) 416–426.
[3] N. Friedreich, F.A. Kekulé, Zur Amyloidfrage, Virchows Archiv fur pathologische Anatomie und Physiologie 16 (1859) 50–65.
[4] F.H. Lewy, Paralysis agitans. I. Pathologische Anatomie, in: M. Lewandowsky (Ed.), Handbuch der Neurologie, Springer, Berlin, 1912, pp. 920–958.
[5] H. Braak, D. Sandmann-Keil, W. Gai, E. Braak, Extensive axonal Lewy neurites in Parkinson's disease: a novel pathological feature revealed by alpha-synuclein immunocytochemistry, Neurosci. Lett. 265 (1999) 67–69.
[6] A.S. Cohen, E. Calkins, Electron microscopic observations on a fibrous component in amyloid of diverse origins, Nature 183 (1959) 1202–1203.
[7] T. Shirahama, A.S. Cohen, High-resolution electron microscopic analysis of the amyloid fibril, J. Cell Biol. 33 (1967) 679–708.
[8] T. Shirahama, A.S. Cohen, Reconstitution of amyloid fibrils from alkaline extracts, J. Cell Biol. 35 (1967) 459–464.
[9] E.D. Eanes, G.G. Glenner, X-ray diffraction studies on amyloid filaments, J. Histochem. Cytochem. 16 (1968) 673–677.
[10] F. Chiti, P. Webster, N. Taddei, A. Clark, M. Stefani, G. Ramponi, C.M. Dobson, Designing conditions for in vitro formation of amyloid protofilaments and fibrils, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3590–3594.
[11] F. Chiti, C.M. Dobson, Protein misfolding, functional amyloid, and human disease, Annu. Rev. Biochem. 75 (2006) 333–366.
[12] L. Maroteaux, J.T. Campanelli, R.H. Scheller, Synuclein—a neuron-specific protein localized to the nucleus and presynaptic nerve-terminal, J. Neurosci. 8 (1988) 2804–2815.
[13] K. Ueda, H. Fukushima, E. Masliah, Y. Xia, A. Iwai, M. Yoshimoto, D.A.C. Otero, J. Kondo, Y. Ihara, T. Saitoh, Molecular-cloning of Cdna-encoding an unrecognized component of amyloid in Alzheimer-disease, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 11282–11286.
[14] R. Jakes, M.G. Spillantini, M. Goedert, Identification of 2 distinct synucleins from human brain, FEBS Lett. 345 (1994) 27–32.
[15] A. Iwai, E. Masliah, M. Yoshimoto, N.F. Ge, L. Flanagan, H.A.R. Desilva, A. Kittel, T. Saitoh, The precursor protein of non-a-beta component of Alzheimers-disease amyloid is a presynaptic protein of the central-nervous-system, Neuron 14 (1995) 467–475.
[16] A. Iwai, M. Yoshimoto, E. Masliah, T. Saitoh, Non-a-beta component of Alzheimers-disease amyloid (Nac) is amyloidogenic, Biochemistry 34 (1995) 10139–10145.
[17] P.H. Weinreb, W.G. Zhen, A.W. Poon, K.A. Conway, P.T. Lansbury, NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded, Biochemistry 35 (1996) 13709–13715.
[18] A. Iwai, E. Masliah, M. Yoshimoto, T. Saitoh, The precursor protein of non-a-beta component of Alzheimers-disease amyloid (Nacp) is a presynaptic protein of the central-nervous-system, Neurobiol. Aging 15 (1994) S58.
[19] A. Iwai, E. Masliah, M.P. Sundsmo, R. Deteresa, M. Mallory, D.P. Salmon, T. Saitoh, The synaptic protein NACP is abnormally expressed during the progression of Alzheimer's disease, Brain Res. 720 (1996) 230–234.
[20] M.H. Polymeropoulos, C. Lavedan, E. Leroy, S.E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, E.S. Stenroos, S. Chandrasekharappa, A. Athanassiadou, T. Papapetropoulos, W.G. Johnson, A.M. Lazzarini, R.C. Duvoisin, G. Di Iorio, L.I. Golbe, R.L. Nussbaum, Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, Science 276 (1997) 2045–2047.
[21] M.G. Spillantini, M.L. Schmidt, V.M. Lee, J.Q. Trojanowski, R. Jakes, M. Goedert, Alpha-synuclein in Lewy bodies, Nature 388 (1997) 839–840.
[22] K. Wakabayashi, K. Matsumoto, K. Takayama, M. Yoshimoto, H. Takahashi, NACP, a presynaptic protein, immunoreactivity in Lewy bodies in Parkinson's disease, Neurosci. Lett. 239 (1997) 45–48.
[23] M.G. Spillantini, R.A. Crowther, R. Jakes, M. Hasegawa, M. Goedert, Alpha-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6469–6473.
[24] K.A. Conway, J.D. Harper, P.T. Lansbury, Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease, Nat. Med. 4 (1998) 1318–1320.
[25] A.B. Singleton, M. Farrer, J. Johnson, A. Singleton, S. Hague, J. Kachergus, M. Hulihan, T. Peuralinna, A. Dutra, R. Nussbaum, S. Lincoln, A. Crawley, M. Hanson, D. Maraganore, C. Adler, M.R. Cookson, M. Muenter, M. Baptista, D. Miller, J. Blancato, J. Hardy, K. Gwinn-Hardy, Alpha-Synuclein locus triplication causes Parkinson's disease, Science 302 (2003) 841.
[26] E. Rockenstein, L.A. Hansen, M. Mallory, J.Q. Trojanowski, D. Galasko, E. Masliah, Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease, Brain Res. 914 (2001) 48–56.
[27] O. Chiba-Falek, G.J. Lopez, R.L. Nussbaum, Levels of alpha-synuclein mRNA in sporadic Parkinson disease patients, Mov. Disord. 21 (2006) 1703–1708.
[28] M.C. Bennett, The role of alpha-synuclein in neurodegenerative diseases, Pharmacol Therapeut. 105 (2005) 311–331.
[29] S. Moussaud, D.R. Jones, E.L. Moussaud-Lamodiere, M. Delenclos, O.A. Ross, P.J. McLean, Alpha-synuclein and tau: teammates in neurodegeneration? Mol. Neurodegener. 9 (2014) 43.
[30] G. Fusco, A. De Simone, T. Gopinath, V. Vostrikov, M. Vendruscolo, C.M. Dobson, G. Veglia, Direct observation of the three regions in alpha-synuclein that determine its membrane-bound behaviour, Nat. Commun. 5 (2014) 3827.
[31] R.A. Crowther, R. Jakes, M.G. Spillantini, M. Goedert, Synthetic filaments assembled from C-terminally truncated alpha-synuclein, FEBS Lett. 436 (1998) 309–312.
[32] T.D. Kim, S.R. Paik, C.H. Yang, Structural and functional implications of C-terminal regions of alpha-synuclein, Biochemistry 41 (2002) 13782–13790.
[33] K. Levitan, D. Chereau, S.I. Cohen, T.P. Knowles, C.M. Dobson, A.L. Fink, J.P. Anderson, J.M. Goldstein, G.L. Millhauser, Conserved C-terminal charge exerts a profound influence on the aggregation rate of alpha-synuclein, J. Mol. Biol. 411 (2011) 329–333.
[34] J.P. Anderson, D.E. Walker, J.M. Goldstein, R. de Laat, K. Banducci, R.J. Caccavello, R. Barbour, J. Huang, K. Kling, M. Lee, L. Diep, P.S. Keim, X. Shen, T. Chataway, M.G. Schlossmacher, P. Seubert, D. Schenk, S. Sinha, W.P. Gai, T.J. Chilcote, Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease, J. Biol. Chem. 281 (2006) 29739–29752.
[35] K.E. Paleologou, A.W. Schmid, C.C. Rospigliosi, H.Y. Kim, G.R. Lamberto, R.A. Fredenburg, P.T. Lansbury, C.O. Fernandez, D. Eliezer, M. Zweckstetter, H.A. Lashuel, Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of alpha-synuclein, J. Biol. Chem. 283 (2008) 16895–16905.
[36] P. Zabrocki, I. Bastiaens, C. Delay, T. Barnmens, R. Ghillebert, K. Pellens, C. De Virgilio, F. Van Leuven, J. Winderickx, Phosphorylation, lipid raft interaction and traffic of alpha-synuclein in a yeast model for Parkinson, Biochim. Biophys. Acta 1783 (2008) 1767–1780.
[37] J.E. Galvin, K. Uryu, V.M. Lee, J.Q. Trojanowski, Axon pathology in Parkinson's disease and Lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13450–13455.
[38] R. Kruger, W. Kuhn, T. Muller, D. Woitalla, M. Graeber, S. Kosel, H. Przuntek, J.T. Epplen, L. Schols, O. Riess, Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease, Nat. Genet. 18 (1998) 106–108.
[39] J.J. Zarranz, J. Alegre, J.C. Gomez-Esteban, E. Lezcano, R. Ros, I. Ampuero, L. Vidal, J. Hoenicka, O. Rodriguez, B. Atares, V. Llorens, E.G. Tortosa, T. del Ser, D.G. Munoz, J.G. de Yebenes, The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia, Ann. Neurol. 55 (2004) 164–173.
[40] P. Pasanen, L. Myllykangas, M. Siitonen, A. Raunio, S. Kaakkola, J. Lyytinen, P.J. Tienari, M. Poyhonen, A. Paetau, A novel alpha-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology, Neurobiol. Aging 35 (2014).

[41] C. Proukakis, C.G. Dudzik, T. Brier, D.S. MacKay, J.M. Cooper, G.L. Millhauser, H. Houlden, A.H. Schapira, A novel alpha-synuclein missense mutation in Parkinson disease, Neurology 80 (2013) 1062–1064.
[42] A.P. Kiely, Y.T. Asi, E. Kara, P. Limousin, H. Ling, P. Lewis, C. Proukakis, N. Quinn, A.J. Lees, J. Hardy, T. Revesz, H. Houlden, J.L. Holton, Alpha-synucleinopathy associated with G51D SNCA mutation: a link between Parkinson's disease and multiple system atrophy? Acta Neuropathol. 125 (2013) 753–769.
[43] R. Kayed, E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, C.G. Glabe, Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, Science 300 (2003) 486–489.
[44] K.M. Danzer, D. Haasen, A.R. Karow, S. Moussaud, M. Habeck, A. Giese, H. Kretzschmar, B. Hengerer, M. Kostka, Different species of alpha-synuclein oligomers induce calcium influx and seeding, J. Neurosci. 27 (2007) 9220–9232.
[45] R. Cappai, S.L. Leck, D.J. Tew, N.A. Williamson, D.P. Smith, D. Galatis, R.A. Sharples, C.C. Curtain, F.E. Ali, R.A. Cherny, J.G. Culvenor, S.P. Bottomley, C.L. Masters, K.J. Barnham, A.F. Hill, Dopamine promotes alpha-synuclein aggregation into SDS-resistant soluble oligomers via a distinct folding pathway, FASEB J. 19 (2005) 1377.
[46] H.A. Lashuel, B.M. Petre, J. Wall, M. Simon, R.J. Nowak, T. Walz, P.T. Lansbury, Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils, J. Mol. Biol. 322 (2002) 1089–1102.
[47] R. Sharon, I. Bar-Joseph, M.P. Frosch, D.M. Walsh, J.A. Hamilton, D.J. Selkoe, The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease, Neuron 37 (2003) 583–595.
[48] T.F. Outeiro, P. Putcha, J.E. Tetzlaff, R. Spoelgen, M. Koker, F. Carvalho, B.T. Hyman, P.J. McLean, Formation of toxic oligomeric alpha-synuclein species in living cells, PloS one 3 (2008) e1867.
[49] J.E. Tetzlaff, P. Putcha, T.F. Outeiro, A. Ivanov, O. Berezovska, B.T. Hyman, P.J. McLean, CHIP targets toxic alpha-synuclein oligomers for degradation, J. Biol. Chem. 283 (2008) 17962–17968.
[50] P. Putcha, K.M. Danzer, L.R. Kranich, A. Scott, M. Silinski, S. Mabbett, C.D. Hicks, J.M. Veal, P.M. Steed, B.T. Hyman, P.J. McLean, Brain-permeable small-molecule inhibitors of Hsp90 prevent alpha-synuclein oligomer formation and rescue alpha-synuclein-induced toxicity, J. Pharmacol. Exp. Ther. 332 (2010) 849–857.
[51] K.M. Danzer, W.P. Ruf, P. Putcha, D. Joyner, T. Hashimoto, C. Glabe, B.T. Hyman, P.J. McLean, Heat-shock protein 70 modulates toxic extracellular alpha-synuclein oligomers and rescues trans-synaptic toxicity, FASEB J. 25 (2011) 326–336.
[52] K.E. Paleologou, C.L. Kragh, D.M. Mann, S.A. Salem, R. Al-Shami, D. Allsop, A.H. Hassan, P.H. Jensen, O.M. El-Agnaf, Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies, Brain 132 (2009) 1093–1101.
[53] M. Andreasen, N. Lorenzen, D. Otzen, Interactions between misfolded protein oligomers and membranes: a central topic in neurodegenerative diseases? Biochim. Biophys. Acta 1848 (2015) 1897–1907.
[54] F.v. Diggelen, A.W.J.W. Tepper, M.M. Apetri, D.E. Otzen, α-Synuclein oligomers: a study in diversity, Israel J. Chem. 57 (2017) 699–723.
[55] A.N. Stefanovic, S. Lindhoud, S.A. Semerdzhiev, M.M. Claessens, V. Subramaniam, Oligomers of Parkinson's disease-related alpha-synuclein mutants have similar structures but distinctive membrane permeabilization properties, Biochemistry 54 (2015) 3142–3150.
[56] N. Lorenzen, S.B. Nielsen, A.K. Buell, J.D. Kaspersen, P. Arosio, B.S. Vad, W. Paslawski, G. Christiansen, Z. Valnickova-Hansen, M. Andreasen, J.J. Enghild, J.S. Pedersen, C.M. Dobson, T.P. Knowles, D.E. Otzen, The role of stable alpha-synuclein oligomers in the molecular events underlying amyloid formation, J. Am. Chem. Soc. 136 (2014) 3859–3868.
[57] N. Zijlstra, C. Blum, I.M. Segers-Nolten, M.M. Claessens, V. Subramaniam, Molecular composition of sub-stoichiometrically labeled alpha-synuclein oligomers determined by single-molecule photobleaching, Angew. Chem. 51 (2012) 8821–8824.
[58] M.J. Volles, S.J. Lee, J.C. Rochet, M.D. Shtilerman, T.T. Ding, J.C. Kessler, P.T. Lansbury Jr., Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease, Biochemistry 40 (2001) 7812–7819.
[59] B.D. van Rooijen, M.M. Claessens, V. Subramaniam, Lipid bilayer disruption by oligomeric alpha-synuclein depends on bilayer charge and accessibility of the hydrophobic core, Biochim. Biophys. Acta 1788 (2009) 1271–1278.
[60] G. Fusco, S.W. Chen, P.T.F. Williamson, R. Cascella, M. Perni, J.A. Jarvis, C. Cecchi, M. Vendruscolo, F. Chiti, N. Cremades, L. Ying, C.M. Dobson, A. De Simone, Structural basis of membrane disruption and cellular toxicity by alpha-synuclein oligomers, Science 358 (2017) 1440–1443.
[61] B.D. van Rooijen, K.A. van Leijenhorst-Groener, M.M. Claessens, V. Subramaniam, Tryptophan fluorescence reveals structural features of alpha-synuclein oligomers, J. Mol. Biol. 394 (2009) 826–833.
[62] M.S. Celej, R. Sarroukh, E. Goormaghtigh, G.D. Fidelio, J.M. Ruysschaert, V. Raussens, Toxic prefibrillar alpha-synuclein amyloid oligomers adopt a distinctive antiparallel beta-sheet structure, Biochem. J. 443 (2012) 719–726.
[63] N. Lorenzen, L. Lemminger, J.N. Pedersen, S.B. Nielsen, D.E. Otzen, The N-terminus of alpha-synuclein is essential for both monomeric and oligomeric interactions with membranes, FEBS Lett. 588 (2014) 497–502.
[64] M. Stockl, M.M. Claessens, V. Subramaniam, Kinetic measurements give new insights into lipid membrane permeabilization by alpha-synuclein oligomers, Mol. BioSyst. 8 (2012) 338–345.
[65] A.N. Stefanovic, M.M. Claessens, C. Blum, V. Subramaniam, Alpha-synuclein amyloid oligomers act as multivalent nanoparticles to cause hemifusion in negatively charged vesicles, Small 11 (2015) 2257–2262.
[66] J.A. Rodriguez, M.I. Ivanova, M.R. Sawaya, D. Cascio, F.E. Reyes, D. Shi, S. Sangwan, E.L. Guenther, L.M. Johnson, M. Zhang, L. Jiang, M.A. Arbing, B.L. Nannenga, J. Hattne, J. Whitelegge, A.S. Brewster, M. Messerschmidt, S. Boutet, N.K. Sauter, T. Gonen, D.S. Eisenberg, Structure of the toxic core of alpha-synuclein from invisible crystals, Nature 525 (2015) 486–490.
[67] M.D. Tuttle, G. Comellas, A.J. Nieuwkoop, D.J. Covell, D.A. Berthold, K.D. Kloepper, J.M. Courtney, J.K. Kim, A.M. Barclay, A. Kendall, W. Wan, G. Stubbs, C.D. Schwieters, V.M. Lee, J.M. George, C.M. Rienstra, Solid-state NMR structure of a pathogenic fibril of full-length human alpha-synuclein, Nat. Struct. Mol. Biol. 23 (2016) 409–415.
[68] L. Bousset, L. Pieri, G. Ruiz-Arlandis, J. Gath, P.H. Jensen, B. Habenstein, K. Madiona, V. Olieric, A. Bockmann, B.H. Meier, R. Melki, Structural and functional characterization of two alpha-synuclein strains, Nat. Commun. 4 (2013) 2575.
[69] M. Vilar, H.T. Chou, T. Luhrs, S.K. Maji, D. Riek-Loher, R. Verel, G. Manning, H. Stahlberg, R. Riek, The fold of alpha-synuclein fibrils, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 8637–8642.
[70] R. Guerrero-Ferreira, N.M.I. Taylor, D. Mona, P. Ringler, M.E. Lauer, R. Riek, M. Britschgi, H. Stahlberg, Cryo-EM structure of alpha-synuclein fibrils, elife 7 (2018).
[71] Y. Li, C. Zhao, F. Luo, Z. Liu, X. Gui, Z. Luo, X. Zhang, D. Li, C. Liu, X. Li, Amyloid fibril structure of alpha-synuclein determined by cryo-electron microscopy, Cell Res. 28 (2018) 897–903.
[72] A. Sidhu, I. Segers-Nolten, V. Subramaniam, Solution conditions define morphological homogeneity of alpha-synuclein fibrils, Biochim. Biophys. Acta 1844 (2014) 2127–2134.
[73] S.J. Roeters, A. Iyer, G. Pletikapic, V. Kogan, V. Subramaniam, S. Woutersen, Evidence for intramolecular antiparallel beta-sheet structure in alpha-synuclein fibrils from a combination of two-dimensional infrared spectroscopy and atomic force microscopy, Sci. Rep. 7 (2017) 41051.
[74] W. Peelaerts, L. Bousset, A. Van der Perren, A. Moskalyuk, R. Pulizzi, M. Giugliano, C. Van den Haute, R. Melki, V. Baekelandt, Alpha-synuclein strains cause distinct synucleinopathies after local and systemic administration, Nature 522 (2015) 340–344.
[75] A.L. Mahul-Mellier, F. Vercruysse, B. Maco, N. Ait-Bouziad, M. De Roo, D. Muller, H.A. Lashuel, Fibril growth and seeding capacity play key roles in alpha-synuclein-mediated apoptotic cell death, Cell Death Differ. 22 (2015) 2107–2122.
[76] H. Chaudhary, V. Subramaniam, M. Claessens, Direct visualization of model membrane remodeling by alpha-synuclein fibrillization, Chemphyschem 18 (2017) 1620–1626.
[77] T. Bartels, J.G. Choi, D.J. Selkoe, Alpha-synuclein occurs physiologically as a helically folded tetramer that resists aggregation, Nature 477 (2011) 107–110.
[78] B. Fauvet, M.K. Mbefo, M.B. Fares, C. Desobry, S. Michael, M.T. Ardah, E. Tsika, P. Coune, M. Prudent, N. Lion, D. Eliezer, D.J. Moore, B. Schneider, P. Aebischer, O.M. El-Agnaf, E. Masliah, H.A. Lashuel, Alpha-synuclein in central nervous system and from erythrocytes, mammalian cells, and Escherichia coli exists predominantly as disordered monomer, J. Biol. Chem. 287 (2012) 15345–15364.
[79] F.X. Theillet, A. Binolfi, B. Bekei, A. Martorana, H.M. Rose, M. Stuiver, S. Verzini, D. Lorenz, M. van Rossum, D. Goldfarb, P. Selenko, Structural disorder of monomeric alpha-synuclein persists in mammalian cells, Nature 530 (2016) 45–50.
[80] W. Wang, I. Perovic, J. Chittuluru, A. Kaganovich, L.T. Nguyen, J. Liao, J.R. Auclair, D. Johnson, A. Landeru, A.K. Simorellis, S. Ju, M.R. Cookson, F.J. Asturias, J.N. Agar, B.N. Webb, C. Kang, D. Ringe, G.A. Petsko, T.C. Pochapsky, Q.Q. Hoang, A soluble alpha-synuclein construct forms a dynamic tetramer, Pro. Natl. Acad. Sci. U. S. A. 108 (2011) 17797–17802.
[81] J. Burre, S. Vivona, J. Diao, M. Sharma, A.T. Brunger, T.C. Sudhof, Properties of native brain alpha-synuclein, Nature 498 (2013) E4–E6 discussion E6–7.
[82] L.B. Lassen, L. Reimer, N. Ferreira, C. Betzer, P.H. Jensen, Protein partners of alpha-synuclein in health and disease, Brain Pathol. 26 (2016) 389–397.
[83] L. Breydo, J.W. Wu, V.N. Uversky, Alpha-synuclein misfolding and Parkinson's disease, Biochim. Biophys. Acta 1822 (2012) 261–285.
[84] D.D. Murphy, S.M. Rueter, J.Q. Trojanowski, V.M.Y. Lee, Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons, J. Neurosci. 20 (2000) 3214–3220.
[85] K.J. Vargas, S. Makani, T. Davis, C.H. Westphal, P.E. Castillo, S.S. Chandra, Synucleins regulate the kinetics of synaptic vesicle endocytosis, J. Neurosci. 34 (2014) 9364–9376.
[86] D. Scott, S. Roy, Alpha-synuclein inhibits intersynaptic vesicle mobility and maintains recycling-pool homeostasis, J. Neurosci. 32 (2012) 10129–10135.
[87] J. Burre, M. Sharma, T. Tsetsenis, V. Buchman, M.R. Etherton, T.C. Sudhof, Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro, Science 329 (2010) 1663–1667.
[88] A. Iyer, S.J. Roeters, N. Schilderink, B. Hommersom, R.M. Heeren, S. Woutersen, M.M. Claessens, V. Subramaniam, The impact of N-terminal acetylation of alpha synuclein on phospholipid membrane binding and fibril structure, J. Biol. Chem. (2016) 21110–21122.
[89] V.V. Shvadchak, L.J. Falomir-Lockhart, D.A. Yushchenko, T.M. Jovin, Specificity and kinetics of alpha-synuclein binding to model membranes determined with fluorescent excited state intramolecular proton transfer (ESIPT) probe, J. Biol. Chem. 286 (2011) 13023–13032.
[90] W.S. Davidson, A. Jonas, D.F. Clayton, J.M. George, Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes, J. Biol. Chem. 273 (1998) 9443–9449.
[91] C.C. Jao, B.G. Hegde, J. Chen, I.S. Haworth, R. Langen, Structure of membrane-bound alpha-synuclein from site-directed spin labeling and computational refinement, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 19666–19671.

[92] V.V. Shvadchak, V. Subramaniam, A four-amino acid linker between repeats in the alpha-synuclein sequence is important for fibril formation, Biochemistry 53 (2014) 279–281.
[93] D. Boassa, M.L. Berlanga, M.A. Yang, M. Terada, J. Hu, E.A. Bushong, M. Hwang, E. Masliah, J.M. George, M.H. Ellisman, Mapping the subcellular distribution of alpha-synuclein in neurons using genetically encoded probes for correlated light and electron microscopy: implications for Parkinson's disease pathogenesis, J. Neurosci. 33 (2013) 2605–2615.
[94] K.J. Spinelli, J.K. Taylor, V.R. Osterberg, M.J. Churchill, E. Pollock, C. Moore, C.K. Meshul, V.K. Unni, Presynaptic alpha-synuclein aggregation in a mouse model of Parkinson's disease, J. Neurosci. 34 (2014) 2037–2050.
[95] L. Wang, U. Das, D.A. Scott, Y. Tang, P.J. McLean, S. Roy, Alpha-synuclein multimers cluster synaptic vesicles and attenuate recycling, Curr. Biol. 24 (2014) 2319–2326.
[96] D.J. Busch, P.A. Oliphint, R.B. Walsh, S.M. Banks, W.S. Woods, J.M. George, J.R. Morgan, Acute increase of alpha-synuclein inhibits synaptic vesicle recycling evoked during intense stimulation, Mol. Biol. Cell 25 (2014) 3926–3941.
[97] M.A. Abolghassemi Fakhree, I.S. Nolten, C. Blum, M. Claessens, Different conformational subensembles of the intrinsically disordered protein alpha-synuclein in cells, J. Phys. Chem. Lett. 9 (2018) 1249–1253.
[98] P.J. McLean, H. Kawamata, S. Ribich, B.T. Hyman, Membrane association and protein conformation of alpha-synuclein in intact neurons - effect of Parkinson's disease-linked mutations, J. Biol. Chem. 275 (2000) 8812–8816.
[99] H.J. Lee, C. Choi, S.J. Lee, Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form, J. Biol. Chem. 277 (2002) 671–678.
[100] M.A. Fakhree, N. Zijlstra, C.C. Raiss, C.J. Siero, H. Grabmayr, A.R. Bausch, C. Blum, M.M. Claessens, The number of alpha-synuclein proteins per vesicle gives insights into its physiological function, Sci. Rep. 6 (2016) 30658.
[101] J. Varkey, J.M. Isas, N. Mizuno, M.B. Jensen, V.K. Bhatia, C.C. Jao, J. Petrlova, J.C. Voss, D.G. Stamou, A.C. Steven, R. Langen, Membrane curvature induction and tubulation are common features of synucleins and apolipoproteins, J. Biol. Chem. 285 (2010) 32486–32493.
[102] A.R. Braun, E. Sevcsik, P. Chin, E. Rhoades, S. Tristram-Nagle, J.N. Sachs, Alpha-synuclein induces both positive mean curvature and negative Gaussian curvature in membranes, J. Am. Chem. Soc. 134 (2012) 2613–2620.
[103] S. Takamori, M. Holt, K. Stenius, E.A. Lemke, M. Gronborg, D. Riedel, H. Urlaub, S. Schenck, B. Brugger, P. Ringler, S.A. Muller, B. Rammner, F. Grater, J.S. Hub, B.L. De Groot, G. Mieskes, Y. Moriyama, J. Klingauf, H. Grubmuller, J. Heuser, F. Wieland, R. Jahn, Molecular anatomy of a trafficking organelle, Cell 127 (2006) 831–846.
[104] D. Eliezer, E. Kutluay, R. Bussell, G. Browne, Conformational properties of alpha-synuclein in its free and lipid-associated states, J. Mol. Biol. 307 (2001) 1061–1073.
[105] S.B. Lokappa, T.S. Ulmer, Alpha-synuclein populates both elongated and broken helix states on small unilamellar vesicles, J. Biol. Chem. 286 (2011) 21450–21457.
[106] C.Y. Cheng, J. Varkey, M.R. Ambroso, R. Langen, S.I. Han, Hydration dynamics as an intrinsic ruler for refining protein structure at lipid membrane interfaces, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 16838–16843.
[107] T.S. Ulmer, A. Bax, N.B. Cole, R.L. Nussbaum, Structure and dynamics of micelle-bound human alpha-synuclein, J. Biol. Chem. 280 (2005) 9595–9603.
[108] T.S. Ulmer, A. Bax, Comparison of structure and dynamics of micelle-bound human alpha-synuclein and Parkinson disease variants, J. Biol. Chem. 280 (2005) 43179–43187.
[109] M. Robotta, P. Braun, B. van Rooijen, V. Subramaniam, M. Huber, M. Drescher, Direct evidence of coexisting horseshoe and extended helix conformations of membrane-bound alpha-synuclein, Chemphyschem 12 (2011) 267–269.
[110] C.R. Bodner, C.M. Dobson, A. Bax, Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy, J. Mol. Biol. 390 (2009) 775–790.
[111] M.E. van Raaij, I.M. Segers-Nolten, V. Subramaniam, Quantitative morphological analysis reveals ultrastructural diversity of amyloid fibrils from alpha-synuclein mutants, Biophys. J. 91 (2006) L96–L98.
[112] S.J. Wood, J. Wypych, S. Steavenson, J.C. Louis, M. Citron, A.L. Biere, Alpha-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease, J. Biol. Chem. 274 (1999) 19509–19512.
[113] A. Iyer, Nils O. Petersen, Mireille M.A.E. Claessens, V. Subramaniam, Amyloids of alpha-synuclein affect the structure and dynamics of supported lipid bilayers, Biophys. J. 106 (2014) 2585–2594.
[114] M. Rabe, A. Soragni, N.P. Reynolds, D. Verdes, E. Liverani, R. Riek, S. Seeger, On-surface aggregation of alpha-synuclein at nanomolar concentrations results in two distinct growth mechanisms, ACS Chem. Neurosci. 4 (2013) 408–417.
[115] N.P. Reynolds, A. Soragni, M. Rabe, D. Verdes, E. Liverani, S. Handschin, R. Riek, S. Seeger, Mechanism of membrane interaction and disruption by alpha-synuclein, J. Am. Chem. Soc. 133 (2011) 19366–19375.
[116] M.M. Ouberai, J. Wang, M.J. Swann, C. Galvagnion, T. Guilliams, C.M. Dobson, M.E. Welland, Alpha-Synuclein senses lipid packing defects and induces lateral expansion of lipids leading to membrane remodeling, J. Biol. Chem. 288 (2013) 20883–20895.
[117] B. Nuscher, F. Kamp, T. Mehnert, S. Odoy, C. Haass, P.J. Kahle, K. Beyer, Alpha-synuclein has a high affinity for packing defects in a bilayer membrane: a thermodynamics study, J. Biol. Chem. 279 (2004) 21966–21975.
[118] E.R. Middleton, E. Rhoades, Effects of curvature and composition on alpha-synuclein binding to lipid vesicles, Biophys. J. 99 (2010) 2279–2288.
[119] K. Pirc, N.P. Ulrih, Alpha-synuclein interactions with phospholipid model membranes: Key roles for electrostatic interactions and lipid-bilayer structure, Biochim. Biophys. Acta 1848 (2015) 2002–2012.
[120] M. Stockl, P. Fischer, E. Wanker, A. Herrmann, Alpha-synuclein selectively binds to anionic phospholipids embedded in liquid-disordered domains, J. Mol. Biol. 375 (2008) 1394–1404.
[121] Y. Zarbiv, D. Simhi-Haham, E. Israeli, S.A. Elhadi, J. Grigoletto, R. Sharon, Lysine residues at the first and second KTKEGV repeats mediate alpha-synuclein binding to membrane phospholipids, Neurobiol. Dis. 70 (2014) 90–98.
[122] E. Jo, J. McLaurin, C.M. Yip, P. St George-Hyslop, P.E. Fraser, Alpha-synuclein membrane interactions and lipid specificity, J. Biol. Chem. 275 (2000) 34328–34334.
[123] E. Rhoades, T.F. Ramlall, W.W. Webb, D. Eliezer, Quantification of alpha-synuclein binding to lipid vesicles using fluorescence correlation spectroscopy, Biophys. J. 90 (2006) 4692–4700.
[124] B.D. van Rooijen, M.M. Claessens, V. Subramaniam, Membrane binding of oligomeric alpha-synuclein depends on bilayer charge and packing, FEBS Lett. 582 (2008) 3788–3792.
[125] A. Stefanovic, Insights into Alpha-Synuclein Oligomer Interactions with Model Membranes, Universiteit Twente, 2014.
[126] A.N.D. Stefanovic, M.T. Stockl, M.M.A.E. Claessens, V. Subramaniam, Alpha-synuclein oligomers distinctively permeabilize model plasma and mitochondrial membranes, Eur. Biophys. J. Biophys. 42 (2013) S156.
[127] A.N.D. Stefanovic, M.T. Stockl, M.M.A.E. Claessens, V. Subramaniam, Alpha-Synuclein oligomers distinctively permeabilize complex model membranes, FEBS J. 281 (2014) 2838–2850.
[128] A. Chaari, H. Horchani, F. Frikha, R. Verger, Y. Gargouri, M. Ladjimi, Surface behavior of alpha-synuclein and its interaction with phospholipids using the Langmuir monolayer technique: a comparison between monomeric and fibrillar alpha-synuclein, Int. J. Biol. Macromol. 58 (2013) 190–198.
[129] E. Hellstrand, A. Nowacka, D. Topgaard, S. Linse, E. Sparr, Membrane lipid co-aggregation with alpha-synuclein fibrils, PLoS One 8 (2013) e77235.
[130] G. Comellas, L.R. Lemkau, D.H. Zhou, J.M. George, C.M. Rienstra, Structural intermediates during alpha-synuclein fibrillogenesis on phospholipid vesicles, J. Am. Chem. Soc. 134 (2012) 5090–5099.
[131] C.M. Pfefferkorn, Z. Jiang, J.C. Lee, Biophysics of alpha-synuclein membrane interactions, Biochim. Biophys. Acta 1818 (2012) 162–171.
[132] H. Chaudhary, A.N. Stefanovic, V. Subramaniam, M.M. Claessens, Membrane interactions and fibrillization of alpha-synuclein play an essential role in membrane disruption, FEBS Lett. 588 (2014) 4457–4463.
[133] R.A. Smith, A. Nabok, B.J. Blakeman, W.F. Xue, B. Abell, D.P. Smith, Analysis of toxic amyloid fibril interactions at natively derived membranes by ellipsometry, PLoS One 10 (2015) e0132309.
[134] A.L. Mahul-Mellier, F. Vercruysse, B. Maco, N. Ait-Bouziad, M. De Roo, D. Muller, H.A. Lashuel, Fibril growth and seeding capacity play key roles in α-synuclein-mediated apoptotic cell death, Cell Death Differ. 22 (2015) 2107–2122.
[135] A.R. Braun, M.M. Lacy, V.C. Ducas, E. Rhoades, J.N. Sachs, Alpha-synuclein-induced membrane remodeling is driven by binding affinity, partition depth, and interleaflet order asymmetry, J. Am. Chem. Soc. 136 (2014) 9962–9972.
[136] A.P. Pandey, F. Haque, J.C. Rochet, J.S. Hovis, Alpha-synuclein-induced tubule formation in lipid bilayers, J. Phys. Chem. B 115 (2011) 5886–5893.
[137] J.C. Stachowiak, E.M. Schmid, C.J. Ryan, H.S. Ann, D.Y. Sasaki, M.B. Sherman, P.L. Geissler, D.A. Fletcher, C.C. Hayden, Membrane bending by protein-protein crowding, Nat. Cell Biol. 14 (2012) 944–949.
[138] D.J. Busch, J.R. Houser, C.C. Hayden, M.B. Sherman, E.M. Lafer, J.C. Stachowiak, Intrinsically disordered proteins drive membrane curvature, Nat. Commun. 6 (2015) 7875.
[139] L. Pieri, K. Madiona, L. Bousset, R. Melki, Fibrillar alpha-synuclein and huntingtin exon 1 assemblies are toxic to the cells, Biophys. J. 102 (2012) 2894–2905.
[140] C.L. Avila, C.M. Torres-Bugeau, L.R. Barbosa, E.M. Sales, M.O. Ouidja, S.B. Socias, M.S. Celej, R. Raisman-Vozari, D. Papy-Garcia, R. Itri, R.N. Chehin, Structural characterization of heparin-induced glyceraldehyde-3-phosphate dehydrogenase protofibrils preventing alpha-synuclein oligomeric species toxicity, J. Biol. Chem. 289 (2014) 13838–13850.
[141] A. Camilleri, C. Zarb, M. Caruana, U. Ostermeier, S. Ghio, T. Hogen, F. Schmidt, A. Giese, N. Vassallo, Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols, Biochim. Biophys. Acta 1828 (2013) 2532–2543.
[142] T. Ryan, V.V. Bamm, M.G. Stykel, C.L. Coackley, K.M. Humphries, R. Jamieson-Williams, R. Ambasudhan, D.D. Mosser, S.A. Lipton, G. Harauz, S.D. Ryan, Cardiolipin exposure on the outer mitochondrial membrane modulates alpha-synuclein, Nat. Commun. 9 (2018) 817.
[143] S. Ghio, F. Kamp, R. Cauchi, A. Giese, N. Vassallo, Interaction of alpha-synuclein with biomembranes in Parkinson's disease–role of cardiolipin, Prog. Lipid Res. 61 (2016) 73–82.
[144] D.L. Fortin, M.D. Troyer, K. Nakamura, S. Kubo, M.D. Anthony, R.H. Edwards, Lipid rafts mediate the synaptic localization of alpha-synuclein, J. Neurosci. 24 (2004) 6715–6723.
[145] L.A. Bagatolli, J.H. Ipsen, A.C. Simonsen, O.G. Mouritsen, An outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes, Prog. Lipid Res. 49 (2010) 378–389.
[146] C. Galvagnion, J.W. Brown, M.M. Ouberai, P. Flagmeier, M. Vendruscolo, A.K. Buell, E. Sparr, C.M. Dobson, Chemical properties of lipids strongly affect the kinetics of the membrane-induced aggregation of alpha-synuclein, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 7065–7070.
[147] E. Hellstrand, M. Grey, M.L. Ainalem, J. Ankner, V.T. Forsyth, G. Fragneto, M. Haertlein, M.T. Dauvergne, H. Nilsson, P. Brundin, S. Linse, T. Nylander, E. Sparr, Adsorption of alpha-synuclein to supported lipid bilayers: positioning

and role of electrostatics, ACS Chem. Neurosci. 4 (2013) 1339–1351.
[148] F. Kamp, K. Beyer, Binding of alpha-synuclein affects the lipid packing in bilayers of small vesicles, J. Biol. Chem. 281 (2006) 9251–9259.
[149] A. Iyer, N. Schilderink, M.M. Claessens, V. Subramaniam, Membrane-bound alpha synuclein clusters induce impaired lipid diffusion and increased lipid packing, Biophys. J. 111 (2016) 2440–2449.
[150] C. Galvagnion, A.K. Buell, G. Meisl, T.C. Michaels, M. Vendruscolo, T.P. Knowles, C.M. Dobson, Lipid vesicles trigger alpha-synuclein aggregation by stimulating primary nucleation, Nat. Chem. Biol. 11 (2015) 229–234.
[151] D. Marsh, Handbook of Lipid Bilayers, 2nd ed., CRC Press, Boca Raton, 2013.
[152] L. Giehm, C.L. Oliveira, G. Christiansen, J.S. Pedersen, D.E. Otzen, SDS-induced fibrillation of alpha-synuclein: an alternative fibrillation pathway, J. Mol. Biol. 401 (2010) 115–133.
[153] C. Galvagnion, The role of lipids interacting with alpha-synuclein in the pathogenesis of Parkinson's disease, J. Park. Dis. 7 (2017) 433–450.
[154] B. Fauvet, M.B. Fares, F. Samuel, I. Dikiy, A. Tandon, D. Eliezer, H.A. Lashuel, Characterization of semisynthetic and naturally nalpha-acetylated alpha-synuclein in vitro and in intact cells: implications for aggregation and cellular properties of alpha-synuclein, J. Biol. Chem. 287 (2012) 28243–28262.
[155] M. Okochi, J. Walter, A. Koyama, S. Nakajo, M. Baba, T. Iwatsubo, L. Meijer, P.J. Kahle, C. Haass, Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein, J. Biol. Chem. 275 (2000) 390–397.
[156] C.E. Ellis, P.L. Schwartzberg, T.L. Grider, D.W. Fink, R.L. Nussbaum, Alpha-synuclein is phosphorylated by members of the Src family of protein-tyrosine kinases, J. Biol. Chem. 276 (2001) 3879–3884.
[157] T. Nakamura, H. Yamashita, T. Takahashi, S. Nakamura, Activated Fyn phosphorylates alpha-synuclein at tyrosine residue 125, Biochem. Biophys. Res. Commun. 280 (2001) 1085–1092.
[158] V.N. Uversky, G. Yamin, P.O. Souillac, J. Goers, C.B. Glaser, A.L. Fink, Methionine oxidation inhibits fibrillation of human alpha-synuclein in vitro, FEBS Lett. 517 (2002) 239–244.
[159] B.I. Giasson, J.E. Duda, I.V. Murray, Q. Chen, J.M. Souza, H.I. Hurtig, H. Ischiropoulos, J.Q. Trojanowski, V.M. Lee, Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions, Science 290 (2000) 985–989.
[160] M. Hasegawa, H. Fujiwara, T. Nonaka, K. Wakabayashi, H. Takahashi, V.M. Lee, J.Q. Trojanowski, D. Mann, T. Iwatsubo, Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions, J. Biol. Chem. 277 (2002) 49071–49076.
[161] V. Dorval, P.E. Fraser, Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein, J. Biol. Chem. 281 (2006) 9919–9924.
[162] A.S. Maltsev, J. Ying, A. Bax, Impact of N-terminal acetylation of alpha-synuclein on its random coil and lipid binding properties, Biochemistry 51 (2012) 5004–5013.
[163] I. Dikiy, D. Eliezer, N-terminal acetylation stabilizes N-terminal helicity in lipid- and micelle-bound alpha-synuclein and increases its affinity for physiological membranes, J. Biol. Chem. 289 (2014) 3652–3665.
[164] N.P. Visanji, S. Wislet-Gendebien, L.W. Oschipok, G. Zhang, I. Aubert, P.E. Fraser, A. Tandon, Effect of Ser-129 phosphorylation on interaction of alpha-synuclein with synaptic and cellular membranes, J. Biol. Chem. 286 (2011) 35863–35873.
[165] K.E. Paleologou, A. Oueslati, G. Shakked, C.C. Rospigliosi, H.Y. Kim, G.R. Lamberto, C.O. Fernandez, A. Schmid, F. Chegini, W.P. Gai, D. Chiappe, M. Moniatte, B.L. Schneider, P. Aebischer, D. Eliezer, M. Zweckstetter, E. Masliah, H.A. Lashuel, Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions, J. Neurosci. 30 (2010) 3184–3198.
[166] C.W. Bertoncini, Y.S. Jung, C.O. Fernandez, W. Hoyer, C. Griesinger, T.M. Jovin, M. Zweckstetter, Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1430–1435.
[167] M. Hejjaoui, S. Butterfield, B. Fauvet, F. Vercruysse, J. Cui, I. Dikiy, M. Prudent, D. Olschewski, Y. Zhang, D. Eliezer, H.A. Lashuel, Elucidating the role of C-terminal post-translational modifications using protein semisynthesis strategies: alpha-synuclein phosphorylation at tyrosine 125, J. Am. Chem. Soc. 134 (2012) 5196–5210.
[168] M.M. Dedmon, K. Lindorff-Larsen, J. Christodoulou, M. Vendruscolo, C.M. Dobson, Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations, J. Am. Chem. Soc. 127 (2005) 476–477.
[169] S. Tenreiro, K. Eckermann, T.F. Outeiro, Protein phosphorylation in neurodegeneration: friend or foe? Front. Mol. Neurosci. 7 (2014) 42.
[170] P. Kumar, N. Schilderink, V. Subramaniam, M. Huber, Membrane binding of Parkinson's protein alpha-synuclein: effect of phosphorylation at positions 87 and 129 by the S to D mutation approach, Israel J. Chem. 57 (2017) 762–770.
[171] A.N. Pronin, A.J. Morris, A. Surguchov, J.L. Benovic, Synucleins are a novel class of substrates for G protein-coupled receptor kinases, J. Biol. Chem. 275 (2000) 26515–26522.
[172] G.S. Nubling, J. Levin, B. Bader, S. Lorenzl, A. Hillmer, T. Hogen, F. Kamp, A. Giese, Modelling Ser129 phosphorylation inhibits membrane binding of pore-forming alpha-synuclein oligomers, PLoS One 9 (2014) e98906.
[173] M. Fiske, S. Valtierra, K. Solvang, M. Zorniak, M. White, S. Herrera, A. Konnikova, R. Brezinsky, S. Debburman, Contribution of alanine-76 and serine phosphorylation in alpha-synuclein membrane association and aggregation in yeasts, Park. Dis. 2011 (2011) 392180.
[174] T. Kuwahara, R. Tonegawa, G. Ito, S. Mitani, T. Iwatsubo, Phosphorylation of alpha-synuclein protein at Ser-129 reduces neuronal dysfunction by lowering its membrane binding property in Caenorhabditis elegans, J. Biol. Chem. 287 (2012) 7098–7109.
[175] S. Azeredo Da Silveira, B.L. Schneider, C. Cifuentes-Diaz, D. Sage, T. Abbas-Terki, T. Iwatsubo, M. Unser, P. Aebischer, Phosphorylation does not prompt, nor prevent, the formation of alpha-synuclein toxic species in a rat model of Parkinson's disease, Hum. Mol. Genet. 18 (2009) 872–887.
[176] A. Oueslati, M. Fournier, H.A. Lashuel, Role of post-translational modifications in modulating the structure, function and toxicity of alpha-synuclein: implications for Parkinson's disease pathogenesis and therapies, Prog. Brain Res. 183 (2010) 115–145.
[177] M.F. Beal, Aging, energy, and oxidative stress in neurodegenerative diseases, Ann. Neurol. 38 (1995) 357–366.
[178] R. Hodara, E.H. Norris, B.I. Giasson, A.J. Mishizen-Eberz, D.R. Lynch, V.M. Lee, H. Ischiropoulos, Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation, J. Biol. Chem. 279 (2004) 47746–47753.
[179] G. Yamin, V.N. Uversky, A.L. Fink, Nitration inhibits fibrillation of human alpha-synuclein in vitro by formation of soluble oligomers, FEBS Lett. 542 (2003) 147–152.
[180] E. Paxinou, Q. Chen, M. Weisse, B.I. Giasson, E.H. Norris, S.M. Rueter, J.Q. Trojanowski, V.M. Lee, H. Ischiropoulos, Induction of alpha-synuclein aggregation by intracellular nitrative insult, J. Neurosci. 21 (2001) 8053–8061.
[181] E. Sevcsik, A.J. Trexler, J.M. Dunn, E. Rhoades, Allostery in a disordered protein: oxidative modifications to alpha-synuclein act distally to regulate membrane binding, J. Am. Chem. Soc. 133 (2011) 7152–7158.
[182] A.S. Maltsev, J. Chen, R.L. Levine, A. Bax, Site-specific interaction between alpha-synuclein and membranes probed by NMR-observed methionine oxidation rates, J. Am. Chem. Soc. 135 (2013) 2943–2946.
[183] M. Baba, S. Nakajo, P.H. Tu, T. Tomita, K. Nakaya, V.M. Lee, J.Q. Trojanowski, T. Iwatsubo, Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies, Am. J. Pathol. 152 (1998) 879–884.
[184] K. Beyer, A. Ariza, Alpha-synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration, Mol. Neurobiol. 47 (2013) 509–524.
[185] A. Iyer, S.J. Roeters, V. Kogan, S. Woutersen, M. Claessens, V. Subramaniam, C-terminal truncated alpha-synuclein fibrils contain strongly twisted beta-sheets, J. Am. Chem. Soc. 139 (2017) 15392–15400.
[186] W. Li, N. West, E. Colla, O. Pletnikova, J.C. Troncoso, L. Marsh, T.M. Dawson, P. Jakala, T. Hartmann, D.L. Price, M.K. Lee, Aggregation promoting C-terminal truncation of alpha-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 2162–2167.
[187] G. Muntane, I. Ferrer, M. Martinez-Vicente, Alpha-synuclein phosphorylation and truncation are normal events in the adult human brain, Neuroscience 200 (2012) 106–119.
[188] C.M. Ritchie, P.J. Thomas, Alpha-synuclein truncation and disease, Health 04 (2012) 1167–1177.
[189] K. Vamvaca, M.J. Volles, P.T. Lansbury Jr., The first N-terminal amino acids of alpha-synuclein are essential for alpha-helical structure formation in vitro and membrane binding in yeast, J. Mol. Biol. 389 (2009) 413–424.
[190] K. Vamvaca, P.T. Lansbury Jr., L. Stefanis, N-terminal deletion does not affect alpha-synuclein membrane binding, self-association and toxicity in human neuroblastoma cells, unlike yeast, J. Neurochem. 119 (2011) 389–397.
[191] T. Bartels, L.S. Ahlstrom, A. Leftin, F. Kamp, C. Haass, M.F. Brown, K. Beyer, The N-terminus of the intrinsically disordered protein alpha-synuclein triggers membrane binding and helix folding, Biophys. J. 99 (2010) 2116–2124.
[192] J. Burre, M. Sharma, T.C. Sudhof, Systematic mutagenesis of alpha-synuclein reveals distinct sequence requirements for physiological and pathological activities, J. Neurosci. 32 (2012) 15227–15242.
[193] K.A. Wilkinson, J.M. Henley, Mechanisms, regulation and consequences of protein SUMOylation, Biochem. J. 428 (2010) 133–145.
[194] P. Krumova, E. Meulmeester, M. Garrido, M. Tirard, H.H. Hsiao, G. Bossis, H. Urlaub, M. Zweckstetter, S. Kugler, F. Melchior, M. Bahr, J.H. Weishaupt, Sumoylation inhibits alpha-synuclein aggregation and toxicity, J. Cell Biol. 194 (2011) 49–60.
[195] E.S. Johnson, Protein modification by SUMO, Ann. Rev. Biochem. 73 (2004) 355–382.
[196] E. Fei, N. Jia, M. Yan, Z. Ying, Q. Sun, H. Wang, T. Zhang, X. Ma, H. Ding, X. Yao, Y. Shi, G. Wang, SUMO-1 modification increases human SOD1 stability and aggregation, Biochem. Biophys. Res. Commun. 347 (2006) 406–412.
[197] M. Kunadt, K. Eckermann, A. Stuendl, J. Gong, B. Russo, K. Strauss, S. Rai, S. Kugler, L. Falomir Lockhart, M. Schwalbe, P. Krumova, L.M. Oliveira, M. Bahr, W. Mobius, J. Levin, A. Giese, N. Kruse, B. Mollenhauer, R. Geiss-Friedlander, A.C. Ludolph, A. Freischmidt, M.S. Feiler, K.M. Danzer, M. Zweckstetter, T.M. Jovin, M. Simons, J.H. Weishaupt, A. Schneider, Extracellular vesicle sorting of alpha-synuclein is regulated by sumoylation, Acta Neuropathol. 129 (2015) 695–713.
[198] G. Fusco, T. Pape, A.D. Stephens, P. Mahou, A.R. Costa, C.F. Kaminski, G.S. Kaminski Schierle, M. Vendruscolo, G. Veglia, C.M. Dobson, A. De Simone, Structural basis of synaptic vesicle assembly promoted by alpha-synuclein, Nat. Commun. 7 (2016) 12563.
[199] M. Robotta, J. Cattani, J.C. Martins, V. Subramaniam, M. Drescher, Alpha-synuclein disease mutations are structurally defective and locally affect membrane binding, J. Am. Chem. Soc. 139 (2017) 4254–4257.
[200] M. Pantusa, B. Vad, O. Lillelund, L. Kjaer, D. Otzen, R. Bartucci, Alpha-synuclein

and familial variants affect the chain order and the thermotropic phase behavior of anionic lipid vesicles, Biochim. Biophys. Acta 1864 (2016) 1206–1214.
[201] P. Flagmeier, G. Meisl, M. Vendruscolo, T.P. Knowles, C.M. Dobson, A.K. Buell, C. Galvagnion, Mutations associated with familial Parkinson's disease alter the initiation and amplification steps of alpha-synuclein aggregation, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 10328–10333.
[202] D. Ysselstein, M. Joshi, V. Mishra, A.M. Griggs, J.M. Asiago, G.P. McCabe, L.A. Stanciu, C.B. Post, J.C. Rochet, Effects of impaired membrane interactions on alpha-synuclein aggregation and neurotoxicity, Neurobiol. Dis. 79 (2015) 150–163.
[203] F. Samuel, W.P. Flavin, S. Iqbal, C. Pacelli, S.D. Sri Renganathan, L.E. Trudeau, E.M. Campbell, P.E. Fraser, A. Tandon, Effects of serine 129 phosphorylation on alpha-synuclein aggregation, membrane association, and internalization, J. Biol. Chem. 291 (2016) 4374–4385.
[204] M.B. Fares, N. Ait-Bouziad, I. Dikiy, M.K. Mbefo, A. Jovicic, A. Kiely, J.L. Holton, S.J. Lee, A.D. Gitler, D. Eliezer, H.A. Lashuel, The novel Parkinson's disease linked mutation G51D attenuates in vitro aggregation and membrane binding of alpha-synuclein, and enhances its secretion and nuclear localization in cells, Hum. Mol. Genet. 23 (2014) 4491–4509.
[205] O. Khalaf, B. Fauvet, A. Oueslati, I. Dikiy, A.L. Mahul-Mellier, F.S. Ruggeri, M.K. Mbefo, F. Vercruysse, G. Dietler, S.J. Lee, D. Eliezer, H.A. Lashuel, The H50Q mutation enhances alpha-synuclein aggregation, secretion, and toxicity, J. Biol. Chem. 289 (2014) 21856–21876.
[206] M. Grey, C.J. Dunning, R. Gaspar, C. Grey, P. Brundin, E. Sparr, S. Linse, Acceleration of alpha-synuclein aggregation by exosomes, J. Biol. Chem. 290 (2015) 2969–2982.
[207] L. Stefanis, Alpha-synuclein in Parkinson's disease, Cold Spring Harbor Perspect. Med. 2 (2012).
[208] M.R. Cookson, Alpha-synuclein and neuronal cell death, Mol. Neurodegener. 4 (2009) 9.
[209] A.A. Cooper, A.D. Gitler, A. Cashikar, C.M. Haynes, K.J. Hill, B. Bhullar, K.N. Liu, K.X. Xu, K.E. Strathearn, F. Liu, S.S. Cao, K.A. Caldwell, G.A. Caldwell, G. Marsischky, R.D. Kolodner, J. Labaer, J.C. Rochet, N.M. Bonini, S. Lindquist, Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models, Science 313 (2006) 324–328.
[210] G. Fiskum, A. Starkov, B.M. Polster, C. Chinopoulos, Mitochondrial mechanisms of neural cell death and neuroprotective interventions in Parkinson's disease, Ann. N. Y. Acad. Sci. 991 (2003) 111–119.
[211] M.C. Quilty, A.E. King, W.P. Gai, D.L. Pountney, A.K. West, J.C. Vickers, T.C. Dickson, Alpha-synuclein is upregulated in neurones in response to chronic oxidative stress and is associated with neuroprotection, Exp. Neurol. 199 (2006) 249–256.
[212] C.E. Ellis, E.J. Murphy, D.C. Mitchell, M.Y. Golovko, F. Scaglia, G.C. Barcelo-Coblijn, R.L. Nussbaum, Mitochondrial lipid abnormality and electron transport chain impairment in mice lacking alpha-synuclein, Mol. Cell. Biol. 25 (2005) 10190–10201.
[213] J. Lotharius, P. Brundin, Pathogenesis of Parkinson's disease: Dopamine, vesicles and alpha-synuclein, Nat. Rev. Neurosci. 3 (2002) 932–942.
[214] T.M. Dawson, V.L. Dawson, Molecular pathways of neurodegeneration in Parkinson's disease, Science 302 (2003) 819–822.
[215] M. Goedert, Alpha-synuclein and neurodegenerative diseases, Nat. Rev. Neurosci. 2 (2001) 492–501.
[216] S.W. Chen, S. Drakulic, E. Deas, M. Ouberai, F.A. Aprile, R. Arranz, S. Ness, C. Roodveldt, T. Guilliams, E.J. De-Genst, D. Klenerman, N.W. Wood, T.P.J. Knowles, C. Alfonso, G. Rivas, A.Y. Abramov, J.M. Valpuesta, C.M. Dobson, N. Cremades, Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation, Proc. Natl. Acad. Sci. 112 (2015) E1994–E2003.
[217] N. Lorenzen, D.E. Otzen, Oligomers of alpha-synuclein: picking the culprit in the line-up, Essays Biochem. 56 (2014) 137–148.
[218] M.H. Horrocks, S.F. Lee, S. Gandhi, M. Iljina, L. Tosatto, C.M. Dobson, D. Klenerman, Single-molecule characterisation of alpha-synuclein oligomers, Biophys. J. 106 (2014) 267a.
[219] C. Fecchio, G. De Franceschi, A. Relini, E. Greggio, M. Dalla Serra, L. Bubacco, P. Polverino De Laureto, Alpha-synuclein oligomers induced by docosahexaenoic acid affect membrane integrity, PloS one 8 (2013) e82732.
[220] K.C. Luk, C. Song, P. O'Brien, A. Stieber, J.R. Branch, K.R. Brunden, J.Q. Trojanowski, V.M. Lee, Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 20051–20056.
[221] C.C. Raiss, T.S. Braun, I.B. Konings, H. Grabmayr, G.C. Hassink, A. Sidhu, J. le Feber, A.R. Bausch, C. Jansen, V. Subramaniam, M.M. Claessens, Functionally different alpha-synuclein inclusions yield insight into Parkinson's disease pathology, Sci. Rep. 6 (2016) 23116.
[222] M. Lee, D.H. Hyun, B. Halliwell, P. Jenner, Effect of the overexpression of wild-type or mutant alpha-synuclein on cell susceptibility to insult, J. Neurochem. 76 (2001) 998–1009.
[223] A.B. Manning-Bog, A.L. McCormack, M.G. Purisai, L.M. Bolin, D.A. Di Monte, Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration, J. Neurosci. 23 (2003) 3095–3099.
[224] M. Hashimoto, L.J. Hsu, E. Rockenstein, T. Takenouchi, M. Mallory, E. Masliah, Alpha-synuclein protects against oxidative stress via inactivation of the c-Jun N-terminal kinase stress-signaling pathway in neuronal cells, J. Biol. Chem. 277 (2002) 11465–11472.
[225] S. Hayashi, K. Wakabayashi, A. Ishikawa, H. Nagai, M. Saito, M. Maruyama, T. Takahashi, T. Ozawa, S. Tsuji, H. Takahashi, An autopsy case of autosomal-recessive juvenile parkinsonism with a homozygous exon 4 deletion in the parkin gene, Movement Disord. 15 (2000) 884–888.
[226] D.J. Moore, A.B. West, V.L. Dawson, T.M. Dawson, Molecular pathophysiology of Parkinson's disease, Annu. Rev. Neurosci. 28 (2005) 57–87.
[227] V. Ruiperez, F. Darios, B. Davletov, Alpha-synuclein, lipids and Parkinson's disease, Prog. Lipid Res. 49 (2010) 420–428.
[228] A.A. Farooqui, L.A. Horrocks, Lipid peroxides in the free radical pathophysiology of brain diseases, Cell. Mol. Neurobiol. 18 (1998) 599–608.
[229] M. Zhu, Z.J. Qin, D.M. Hu, L.A. Munishkina, A.L. Fink, Alpha-synuclein can function as an antioxidant preventing oxidation of unsaturated lipid in vesicles, Biochemistry 45 (2006) 8135–8142.
[230] F. Zoccarato, P. Toscano, A. Alexandre, Dopamine-derived dopaminochrome promotes H2O2 release at mitochondrial complex I—stimulation by rotenone, control by Ca2+, and relevance to Parkinson disease, J. Biol. Chem. 280 (2005) 15587–15594.
[231] K.A. Conway, J.C. Rochet, R.M. Bieganski, P.T. Lansbury Jr., Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct, Science 294 (2001) 1346–1349.
[232] Z. Qin, D. Hu, S. Han, S.H. Reaney, D.A. Di Monte, A.L. Fink, Effect of 4-hydroxy-2-nonenal modification on alpha-synuclein aggregation, J. Biol. Chem. 282 (2007) 5862–5870.
[233] R.E. Colebrooke, T. Humby, P.J. Lynch, D.P. McGowan, J. Xia, P.C. Emson, Age-related decline in striatal dopamine content and motor performance occurs in the absence of nigral cell loss in a genetic mouse model of Parkinson's disease, Eur. J. Neurosci. 24 (2006) 2622–2630.
[234] V. Loeb, E. Yakunin, A. Saada, R. Sharon, The transgenic overexpression of alpha-synuclein and not its related pathology associates with complex I inhibition, J. Biol. Chem. 285 (2010) 7334–7343.
[235] S. Kaul, V. Anantharam, A. Kanthasamy, A.G. Kanthasamy, Wild-type alpha-synuclein interacts with pro-apoptotic proteins PKCdelta and BAD to protect dopaminergic neuronal cells against MPP+−induced apoptotic cell death, Brain Res. Mol. Brain Res. 139 (2005) 137–152.
[236] M.S. Parihar, A. Parihar, M. Fujita, M. Hashimoto, P. Ghafourifar, Alpha-synuclein overexpression and aggregation exacerbates impairment of mitochondrial functions by augmenting oxidative stress in human neuroblastoma cells, Int. J. Biochem. Cell B. 41 (2009) 2015–2024.
[237] M.S. Parihar, A. Parihar, M. Fujita, M. Hashimoto, P. Ghafourifar, Mitochondrial association of alpha-synuclein causes oxidative stress, Cell. Mol. Life Sci. 65 (2008) 1272–1284.
[238] A.R. Giniatullin, F. Darios, A. Shakirzyanova, B. Davletov, R. Giniatullin, SNAP25 is a pre-synaptic target for the depressant action of reactive oxygen species on transmitter release, J. Neurochem. 98 (2006) 1789–1797.
[239] F. Kamp, N. Exner, A.K. Lutz, N. Wender, J. Hegermann, B. Brunner, B. Nuscher, T. Bartels, A. Giese, K. Beyer, S. Eimer, K.F. Winklhofer, C. Haass, Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1, EMBO J. 29 (2010) 3571–3589.
[240] K. Nakamura, V.M. Nemani, F. Azarbal, G. Skibinski, J.M. Levy, K. Egami, L. Munishkina, J. Zhang, B. Gardner, J. Wakabayashi, H. Sesaki, Y.F. Cheng, S. Finkbeiner, R.L. Nussbaum, E. Masliah, R.H. Edwards, Direct membrane association drives mitochondrial fission by the Parkinson disease-associated protein alpha-synuclein, J. Biol. Chem. 286 (2011) 20710–20726.